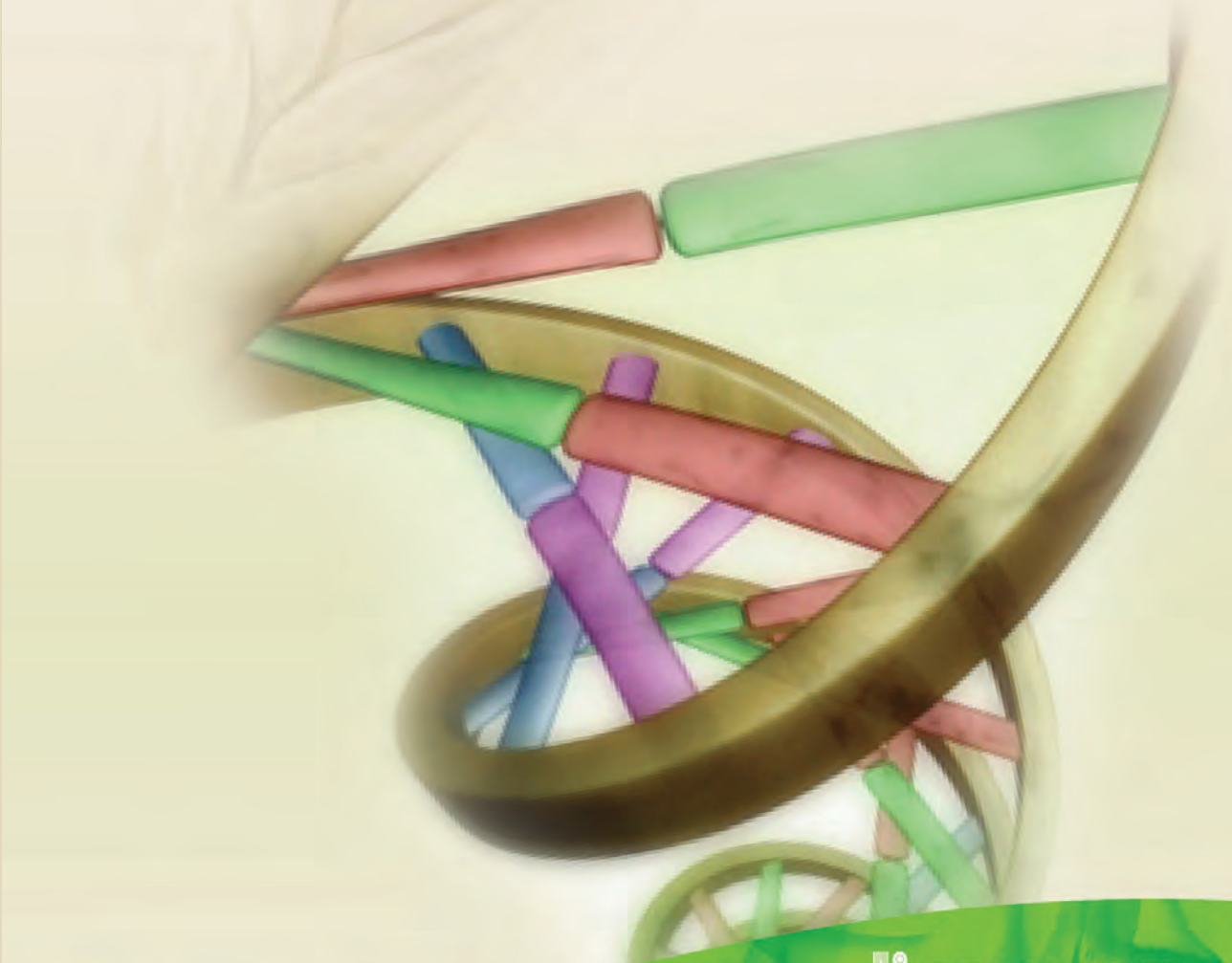
BOOK OF ABSTRACTS

21st International Triticeae Mapping Initiative

WORKSHOP

Susanne Dreisigacker and Sukhwinder Singh, Editors

September 5 - 9, 2011 - Mexico City, Mexico



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21st International Triticeae Mapping Initiative Workshop

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5-9 September 2011
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EDITORS:
Dr. Susanne Dreisigacker
Dr. Sukhwinder Singh

With valuable editorial assistance from:
Dr. Etienne Duveiller
Dr. David Bonnett
Daniela Flores
Maribel Velázquez
Liliana Rodríguez
Ma. Teresa Rodríguez

EDITORIAL OFFICE:

International Maize and Wheat Improvement Center (CIMMYT, Int.)
Apdo. Postal 6-641, 06600, Mexico, D.F.
Office: +52 55 28 04 2004
Fax: +52 55 5804 7458
Directly from the USA: +1 650 833 6655

Design and Production: Miguel Mellado, Eliot Sánchez, and Marcelo Ortiz

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Session 4: Exploring and exploiting Triticeae Genetic Resources

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Session 5: Bioinformatics and Computational Biology

David Marshall	**Chair-** Scottish Crop Research Institute	United Kingdom
Jiankang Wang	International Maize and Wheat Improvement Center (CIMMYT)	Mexico
Frederic Choulet	Institute for Agricultural Research (INRA)	France
Mario Caccamo	The Genome Analysis Centre (TGAC)	United Kingdom

Session 6: New initiatives and technologies

Eduard Akhunov	**Chair -** Kansas State University	USA
Andrzej Kilian	Diversity Arrays Technology Pty Ltd	Australia
Huw Jones	Rothamsted	United Kingdom

PROGRAM

Sunday 4 September

08:00 – 10:00	IWGSC & ITMI - Workshop registration Place: In front of "Sala Murillo" (Floor # 4)
10:00 – 17:00	IWGSC pre-conference workshop Place: Sala Murillo
16:00 – 18:00	ITMI – Workshop registration Place: In front of "Sala Murillo"
19:00 – 21:00	Welcome cocktail Place: Pent house

Monday 5 September

08:00 – 09:00	Workshop registration
	Welcome
09:00 – 09:10	Dr. Thomas A. Lumpkin, Director, CIMMYT, Mexico
09:10 – 09:20	Dr. José Fernando De La Torre Sánchez, Director, Nacional Center of Genetic Resources, INIFAP, México
09:20 – 09:30	Dr. Catherine Feuillet, ITMI-chair, INRA, France
	Opening lectures
09:30 – 10:20	Hans-J. Braun, Global Wheat Program, CIMMYT, Mexico Title: The challenges for global wheat production –1 billion tons by 2050
10:20 – 10:50	Coffee break
10:50– 11:40	Jorge Dubcovsky, UC Davis, USA Title: Gene networks regulating flowering time in wheat
11:40 – 12:30	Takao Komatsuda, NIAS, Japan Title: The role of gene duplication in the domestication of barley
12:30 – 14:00	Lunch
	Session 1: Structural and Functional Genomics
14:00 – 14:40	Invited Speaker: Catherine Feuillet, INRA, France Title: Towards A Reference Sequence of Wheat Chromosome 3B
14:40 – 15:10	Speaker: Ming-Cheng Luo, University of California, USA Title: Genomics of *Aegilops tauschii*: The current status
15:10 – 15:40	Speaker: Nils Stein, IPK Gatersleben, Germany Title: The barley genome – from virtual gene order to a genetically anchored physical map
15:40 – 16:00	Coffee break
16:00 – 16:30	Speaker: Mario Caccamo, The Genome Analysis Centre (TGAC), UK Title: Bread Wheat Chromosome-based Survey Sequencing Initiative
16:30 – 17:00	Speaker: Kunal Mukhopadhyay, Birla Institute of Technology, India Title: Molecular dissection of wheat transcriptome using next-generation SOLiD sequencing technology during compatible and incompatible wheat-leaf rust interaction.
18:00 – 19:30	International Research Initiative for Wheat Improvement – Introduction (Hélèn Lucas, Hans-J. Braun) and discussion forum

Tuesday 6 September

09:00 – 9:30	Speaker: Pierre Sourdille, INRA, France Title: Identification and location of chromosome-pairing promoters on chromosome 3B of bread wheat
09:30 – 10:00	Speaker: Tamar Krugman Title: Shift in expression of hormonal-related genes result in enhance drought tolerance in wild emmer wheat
	Session 2: Mapping and Cloning
10:00 – 10:40	Invited Speaker: Xiue Wang, Nanjing Agricultural University, China Title: Towards the fine mapping and cloning the disease resistance genes from different sources in wheat
10:40 – 11:00	Coffee break
11:00 – 11:30	Speaker: María José Diéguez, INTA, Argentina Title: Towards positional cloning of SV2, an specific adult plant wheat leaf rust resistance gene identified in the durable resistant variety Sinvalocho MA
11:30 – 12:00	Speaker: Freddy Yeo, Wageningen University, Netherlands Title: Low homology in the neighborhood of Resistance to *Puccinia hordei* QTL2
12:00 – 12:30	Speaker: Hana Simkova, Centre of the Region Hana, Czech Republic Title: Chromosome genomics facilitates cloning of a Russian wheat aphid resistance gene
12:30 – 14:00	Lunch
14:00 – 14:30	Speaker: Bao Lam Huynh, ACPFG, Australia Title: Positional cloning and analysis of genes coding for fructan biosynthesizing enzymes in wheat and barley
14:30 – 15:00	Speaker: Saintenac Cyrille, Kansas State University, USA Title: Map based cloning and characterization of Ug99 resistance gene *Sr35*
15:00 – 15:30	Speaker: Margarita Shatalina, University of Zürich, Switzerland Title: Using sequence of flow-sorted chromosome 3B to develop SNP and InDel markers for genetic mapping
15:30 – 16:00	Coffee break
16:00 – 18:00	Poster session Place: Sala Murillo
18:00 – 19:00	*IWGSC – consortium meeting (closed for consortium members)*

Wednesday 7 September

06:30 – 13:00	Field day – Toluca Experimental Station
13:00 – 14:30	Lunch
	Session 3: How to bridge potential and practice? Applied Molecular Breeding
14:30 – 15:10	Invited Speaker: Viktor Korzun, KWS Lochow, Germany Title: Molecular breeding in cereals: current status and perspectives
15:10 – 15:40	Speaker: Eric Storlie, INRA, France Title: Predicting Wheat Yield with Markers

15:40 – 16:00	Coffee break
16:00 – 16:30	Speaker: Yann Manès, CIMMYT, Mexico Title: Genomic prediction of breeding traits in bread wheat
16:30 – 17:00	Speaker: Herman Buerstmayr, BOKU, Austria Title: Marker assisted breeding for improving Fusarium head blight resistance in wheat

Thursday 8 September

09:00 – 09:30	Speaker: Colin Cavanagh, CSIRO, Australia Title: Using a Multi-parent genetic population to generate a high resolution SNP genetic map and exploring the underlying genetic architecture
09:30 – 10:00	Speaker: Beat Keller, University of Zürich, Switzerland Title: Molecular breeding using transgenic wheat: a case study for fungal disease resistance
10:00 – 10:30	Speaker: Hirokazu Handa, National Institute of Agrobiological Sciences, Japan Title: Characterization of a wheat transcription factor, *TaWRKY45*, and its overexpression confers multiple fungal disease resistances in transgenic wheat plants
10:30 – 10:50	Coffee break
	Session 4: Exploring and exploiting Triticeae Genetic Resources
10:50 – 11:30	Invited Speaker: Shuhei Nasuda, Kyoto University, Japan Title: National BioResource Project-Wheat, Japan: Conservation and Utilization of Genetic Resources of Wheat
11:30 – 12:00	Speaker: Nicolas Gosman, NIAB, UK Title: Comparative analysis of D-genome diversity in *Aegilops tauschii*, common bread wheat (*Triticum aestivum*) & synthetic hexaploid wheat
12:00 – 12:30	Speaker: Simon Krattinger, CSIRO, Australia Title: *Lr34* multi-pathogen resistance ABC transporter: molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species
12:30 – 13:30	Lunch
13:30 – 14:00	Speaker: Herman Buerstmayr, BOKU, Austria Title: Advanced back-cross QTL mapping of resistance to Fusarium head blight derived from *Triticum macha* (Georgian spelt wheat)
14:00 – 14:30	Speaker: Francis Ogbonnaya, ICARDA, Syria Title: The application of molecular markers for wheat improvement: Breeding for stem rust resistance in Kazakhstan
14:30 – 15:00	Speaker: Martin Ganal, TraitGenetics GmbH, Germany Title: SNP and haplotype identification in a large set of wheat genes
15:00 – 15:30	Speaker: Cynthia Taylor Lawley, Illumina, USA Title: Refining software tools for calling genotypes using high density SNP panels (>10,000 markers) in polyploid species
15:45	Departure: Muesum Templo Mayor

19:00 – 21:00	Mexican Fiesta Dinner: Terrace "Majestic" Place: Historical Center, Mexico City

Friday 9 September

	Session 5: Bioinformatics and Computational Biology
09:00 – 09:40	Invited Speaker: Hadi Quesneville, URGI, France Title: High-throughput data integration at URGI
09:40 – 10:10	Speaker: Manuel Spannagl, Helmholtz Zentrum, Germany Title: Alphabet soup: some bioinformatic recipes for Triticeae
10:10 – 10:40	Speaker: Jiankang Wang, CAAS, China Title: QTL IciMapping v3.1: Integrated Software for Building Linkage Maps and Mapping Quantitative Trait Genes
10:40 – 11:00	Coffee break
11:00 – 11:30	Speaker: Hiroaki Sakai, National Institute of Agrobiological Sciences, Japan Title: Comprehensive analyses of 24,783 barley full-length cDNAs derived from 12 clone libraries
11:30 – 11:40	Speaker: Paul Brenan, CropGen International, Australia Title: GENESYS – A Gateway to plant genetic resources
	Session 6: New initiatives and technologies
11:40 – 12:10	Invited Speaker: Peter Wenzl, CIMMYT, Mexico Title: SeeD: A Mexican initiative to characterize and broaden the genetic base of maize and wheat
12:10 – 12:40	Speaker: Kelly Eversole, IWGSC, USA Title: The International Wheat Genome Sequencing Consortium (IWGSC): Building the Foundation for a Paradigm Shift in Wheat Breeding
12:40 – 14:00	Lunch
14:00 – 14:30	Speaker: Jesse Poland, USDA, USA Title: Genotyping-by-sequencing for barley and wheat breeding and genetics
14:30 – 15:00	Speaker: Paux Etienne, INRA, France Title: High throughput marker development from the repetitive fraction of the wheat genome
15:00 – 15:30	Speaker: Eduard Akhunov, Kansas State University, USA Title: Next-generation tools for wheat genetics and breeding: high-throughput SNP genotyping assays and sequence-based genotyping
15:30 – 16:00	Speaker: Martin Ganal, TraitGenetics GmbH, Germany Title: A 9K Infinium array for the characterization of barley lines and genetic mapping
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Opening Lectures

The challenges for global wheat production – 1billion tons by 2050

H-J. Braun

International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico DF, Mexico

Recurrent food crises—combined with the global financial meltdown, volatile energy prices, natural resource depletion, and climate change—undercut and threaten the livelihoods of millions of poor people. Accounting for a fifth of humanity's food, wheat is second only to rice as a source of calories in the diets of developing country consumers, and it is first as a source of protein. Wheat is an especially critical "staff of life" for the approximately 1.2 billion "wheat dependent" to 2.5 billion "wheat consuming" poor—men, women and children who live on less than USD 2 per day—and approximately 30 million poor wheat producers and their families. Demand for wheat in the developing world is projected to increase 60% by 2050. At the same time, climate-change-induced temperature increases are likely to reduce wheat production in developing countries by 20–30%. As a result, prices will more than double in real terms, eroding the purchasing power of poor consumers and creating conditions for widespread social unrest. This scenario is worsened by stagnating yields, soil degradation, increasing irrigation and fertilizer costs, and virulent new disease and pest strains. The world wheat research community has to implement new, result-oriented strategies through a series of programs to fully exploit the potential of international agricultural research for development to enhance global food security and environmental sustainability.

Gene networks regulating flowering time in wheat

J. Dubcovsky, C. Li, A. Chen

Department of Plant Sciences, University of California, Davis, California, USA

A precise regulation of the transition from vegetative to reproductive development is essential to maximize wheat productivity in changing environments. This transition is mainly regulated by seasonal cues including vernalization (determined mainly by *VRN1* and *VRN2*) and photoperiod (determined mainly by *PPD1* and *CO2*). The wheat VRN3, which is similar to Arabidopsis FT, plays a central role in the integration of the signals from these two pathways. Under long days, *VRN3* transcription is down-regulated by VRN2 and up-regulated by CO2. Overexpression of *VRN3* overcomes VRN2 repression and promotes *VRN1* transcription and flowering initiation. Using yeast two-hybrid assays we found that the CCT domains present in VRN2 and CO2 proteins interact with several NF-Y proteins. NF-Y proteins form trimeric complexes including NF-YA, NF-YB and NF-YC, which then interact with CCAAT *cis* regulatory elements in the promoters of many eukaryotic genes (e.g. *FT*). Interestingly, VRN2 and CO2 proteins interact with the same subset of eight NF-Y proteins suggesting that they may compete for these same interactors. Using yeast three-hybrid assays we demonstrated that VRN2 and CO2 CCT proteins compete with NF-YA (and with each other) for interactions with NF-YB proteins. A conserved region of the NF-YA protein and the CCT domain share several conserved amino acids, suggesting a common ancestral origin. Mutations in these conserved amino acids in the CCT domain of VRN2 protein that eliminate the vernalization requirement in winter wheat also reduce the strength of the interactions between VRN2 and NF-Y proteins, and the ability of VRN2 to compete with CO2. Taken together, our results suggest that the interactions between CCT and NF-Y proteins play an important role in the integration of the vernalization and photoperiod seasonal signals, and provide a flexible combinatorial system to integrate multiple developmental and environmental signals in wheat regulation of flowering initiation. The expansion of the *NF-Y* and CCT domain proteins in plants has created the potential for a huge number of different trimeric NF-Y and NF-Y/CCT complexes, providing a flexible system to accommodate the many developmental and environmental signals that are integrated in flowering regulation. Since several *NF-Y* genes are transcriptionally regulated by abiotic stresses, the NF-Y–CCT interactions may provide a central hub to integrate seasonal cues with multiple stress signals in the regulation of flowering time.

The role of gene duplication in the domestication of barley

T. Komatsuda[1, 2], S. Sakuma[1, 2], S. Ning[1, 2], N. Wang[1], M. Pourkheirandish[1], T. Matsumoto[1]

[1]National Institute of Agrobiological Sciences, Tsukuba, Japan
[2]Graduate School of Horticulture, Chiba University, Matsudo, Japan

The non-brittle rachis is one of several major domestication traits in barley (*Hordeum vulgare* subsp. *vulgare*), since without it, the spike shatters at maturity, thereby preventing the grain from being readily harvested. Early farmers would therefore have tended to impose a heavy selection pressure in favour of non-brittle types. The six-rowed spike is a second key domestication trait, given its potential to set three times as many grains per spike as the wild type two-rowed form. The canonical arrangement of three spikelets attached to each rachis node (one central and two lateral spikelets) is characteristic of the *Hordeum* genus. In cultivated barley, row type is determined by allelic constitution at the *Vrs1* locus, present on the long arm of chromosome 2H; the dominant allele encodes a homeodomain-leucine zipper I protein, while a number of both naturally occurring and induced mutant recessive alleles are responsible for the formation of the six-rowed spike. The gene is thought to have evolved via the duplication of a paralogous locus (*HvHox2*) mapping to the short arm of chromosome 2H. A phylogenetic analysis has demonstrated that the duplication affected the common ancestor of the Triticeae species, but is not found in other Poaceae species whose genome sequence is currently available. *HvHox2* is expressed throughout the plant, while the dominant allele at *Vrs1* is only transcribed in the lateral spikelets. We suggest that this localized expression of *Vrs1* suppresses the formation of the lateral spikelets, because the VRS1 and HvHOX2 proteins both may share the same target DNA sequence with each having retained an equivalent level of binding affinity. The simultaneous presence of the *Vrs1* and *HvHox2* products would allow for the creation of an HvHOX2/VRS1 heterodimer, which would reduce the frequency of HvHOX2 homodimers present. The VRS1 protein differs from HvHOX2 by the absence of an eight residue motif at the C terminus of the protein, which may act as an activator. Our hypothesis is that *Vrs1* acquired the capacity to suppress *HvHox2* during the evolution of the barley and its close relatives. An equivalent scenario (gene duplication followed by a change of function of one copy) has been proposed for the *HvAP2* and *HvAP2-like* genes. Here, a single nucleotide substitution at the microRNA targeting site in *HvAp2* produces a recessive allele responsible for cleistogamy and the formation of a dense spike. This mutation appears to be a relatively recent event in the evolution of barley.

Oral Presentations

Session 1
Structural and Funtional Genomics

Towards A Reference Sequence of Wheat Chromosome 3B

F. Choulet[1], S. Theil[1], N. Guilhot[1], A. Bernard[1], A. Couloux[2], A. Alberti[2], M. Alaux[3], P. Leroy[1], H. Šimková[4], J. Doležel[4], A. Bellec[5], H. Bergès[5], P. Sourdille[1], E. Paux[1], H. Quesneville[3], P. Wincker[2], C. Feuillet[1]

[1]INRA-University Blaise Pascal Joint Research Unit 1095 Genetics, Diversity and Ecophysiology of Cereals, Clermont-Ferrand 63100, France
[2]Genoscope, Institute of Genomics, CEA, Evry 91057, France
[3]INRA Research Unit 1164 Genomics-Informatics, Versailles 78026, France
[4]Institute of Experimental Botany, Olomouc 77200, Czech Republic
[5]INRA French Plant Genomic Resource Centre, Castanet Tolosan 31326, France

The production of a reference sequence of the hexaploid wheat chromosome 3B is currently underway in the framework of a ANR-France Agrimer funded project (3BSEQ, http://urgi.versailles.inra.fr/Projects/3BSeq). To cover the 1000 Mb, a total of 8441 BAC clones representing the minimal tiling path (MTP) of the 3B physical map was sequenced using Roche-454 Titanium technology. Barcoded pools of about 10 BACs each are sequenced *de novo*. Ideally, pooled BACs belong to the same physical contig and, thus, share overlapping inserts. However, half of the pools contain BAC from two or more different contigs. A 8 kb mate-pair library is built for each pool to ensure the assembly of large scaffolds which is a major criteria to reach a high quality reference sequence of 3B. In addition, about 40'000 BAC-end sequences were produced and a 50X coverage of sorted 3B chromosomes was also generated by Illumina sequencing. First pool assemblies revealed a scaffold N50 at 220 kb. In addition, for 80% of the BAC pools, less than 5 scaffolds cover 90% of the sequenced region, revealing that the assembled sequence is of high quality. Sequence quality was evaluated by comparing *de novo* assemblies with reference contig sequences generated previously (Choulet el al, 2010). This revealed that genes are correctly assembled and fully included into large sequence contigs, suggesting that the whole 3B gene space will be assessed through this approach. Moreover, even though some TE rich regions still contain large gaps because of unresolved read pairs, scaffolds are properly assembled and the size of the gaps is correctly estimated. Assembly parameters were optimized leading to a significant increase of the quality of the assembly for most of the BAC pools. In parallel, a new version of the annotation pipeline TriAnnot that enables parallel computing and rapid annotation has been established. Results about sequencing, assembling and annotation will be discussed.

Genomics of *Aegilops tauschii*: The current status

M.C Luo[1], S. Chen[1], K.R. Deal[1], Y. Hu[1], Y. Ma[1], P.E. McGuire[1], J. Wang[1], F.M. You[1], N. Huo[2], Y.Q. Gu[2], O.D. Anderson[2], W. Li[3], S.K. Sehgal[3], B.S Gill[3], J. Dolezel[4], J.C. Stein[5]; S. Pasternak[5,6], A. Olson[5], D. Ware[5], R. McCombie[5], M. Martis[7], K. Mayer[7], J. Dvorak[1]

[1]Department of Plants Sciences, University of California, Davis, CA 95616 USA
[2]U.S. Department of Agriculture, ARS-WRRC, 800 Buchanan St., Albany, CA 94710, USA
[3]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506 USA
[4]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovska 6, CZ-77200 Olomouc, Czech Republic
[5]CSHL, Cold Spring Harbor, NY 11724 USA
[6]U.S. Department of Agriculture, ARS, NAA Robert W. Holley Center for Agriculture and Health, Cornell University, Ithaca, NY 14853 USA
[7]Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany

Aegilops tauschii is the diploid source of the wheat D genome and an important resource for wheat genetics and genomics. A 50X genome equivalent of Illumina reads, 3.1X genome equivalent of Roche 454 reads, 20X genome equivalent of SOLiD genomic reads, and SOLiD cDNA reads of leaf, crown and root tissues were generated and used in the assembly of gene-space sequences of *Ae. tauschii* accession AL8/78 collected by V. Jaaska in Armenia. A bacterial artificial chromosome (BAC) clone-based physical map of the *Ae. tauschii* (AL8/78) genome is being constructed to facilitate complete sequencing of *Ae. tauschii* genome using the ordered clone approach. Towards this goal, 598,872 clones fingerprinted with the SNaPshot method from nine libraries were assembled into 3,516 contigs. The contigs are being ordered with the assistance of a high-density genetic map of *Ae. tauschii*. To generate a sufficient number of SNPs for the construction of the genetic map, a pipeline (AGSNP) for global SNP discovery without a prerequisite genome sequence was developed. A total of 195,631 gene-based SNPs between the parents of the mapping population (AL8/78 and AS75) were discovered and Illumina's 10K Infinium was designed and used to genotype the *Ae. tauschii* mapping population of 1,102 F_2 plants. The resulting genetic map contains 6,980 gene loci. The map is being used as a framework to generate an *Ae. tauschii* genome zipper and for anchoring BAC contigs for the construction of the physical map. A minimum tiling path of 42,561 clones across the contigs was constructed for the future sequencing of the *Ae. tauschii* genome by the ordered clone approach.

The barley genome – from virtual gene order to a genetically anchored physical map

N. Stein

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany

The International Barley Genome Sequencing Consortium set out for physical mapping and sequencing the barley genome in 2006. This was just at the time when next generation sequencing platforms became also established for applications in the analysis of complex genomes comprised of mainly by repetitive DNA. Since then genomic resources have been established that make sequencing the barley genome a realistic undertaken. Recently, a high resolution global view on gene content and virtual gene order of the barley genome could be revealed by chromosomal genomics – survey sequencing of sorted barley chromosomes (Mayer et al. 2011, The Plant Cell 23:1249-1263). In parallel a physical map is under construction by High Information Content Fingerprinting of 14-fold genome coverage of BAC clones. Anchoring of the map to the genetic map is in progress which takes advantage also of the virtual gene order information now available for barley. An overview of the current status of the work will be provided.

Bread Wheat Chromosome-based Survey Sequencing Initiative

M. Caccamo[1] on behalf of the International Wheat Genome Sequencing Consortium[2]

[1]Head of Bioinformatics, The Genome Analysis Centre, Norwich Research Park, NR4 7UH Norwich, United Kingdom
[2]International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org)

Grass crops, and in particular bread wheat (*Triticum aestivum)*, contribute directly as one of the main sources for human and animal nutrition. Applying advanced genomics to wheat breeding techniques will play a central role in securing affordable and nutritious food. Bread wheat, however, has one of the largest (~17Gb) and most complex (allohexaploid, 6n=42, AA,BB,DD) genomes. Although genome size varies in grasses due to the expansion of retroelements, gene order, generally, is conserved along large chromosomal segments enabling comparative methods between related species. The IWGSC aims to establish a high quality, gold-standard reference sequence of the wheat genome anchored to the genetic maps that will provide high resolution links between the traits and the underlying variations in sequence and polymorphisms. To deliver tools rapidly while working towards the ultimate goal, the IWGSC implements short- and long-term strategic roadmaps. The first goal has been to obtain physical maps of the individual chromosome arms for the reference variety Chinese Spring. To anchor the physical contigs and provide useful sequencing data immediately to the breeding community, survey sequences (40-80x) were achieved using next-generation sequencing technologies for more than half of the chromosomes. To complete this effort, the IWGSC launched a short-term initiative to provide low coverage, survey sequences of all 21 wheat chromosomes. Sponsored by Graminor, Biogemma, INRA, and the Institute of Experimental Biology, the aim is to generate sequence and virtual order for most wheat genes. The sequences generated for each chromosome arm are assembled using the latest software tools. One of the main challenges of this stage is the repeat content and the size of the target (~500Mb for the largest chromosome arms). A first pass annotation is implemented over the draft assembly sequences where comparative genomics and colinearity with other grass genomes is used to derive a virtual gene order with an account of non-syntenic genes and pseudogenes. As sequences are generated independently for each chromosome, a second aim of this project is to characterise genomic variation between gene homoeologues (ie orthologous genes placed in different subgenomes), regulatory elements and repeat content. An update of this initiative will be presented.

Dissection of wheat transcriptome using next-generation SOLiD sequencing technology during compatible and incompatible wheat-leaf rust interaction

D. Singh[1], G. Bagnagre[2], R. Bandopadhyay[1], K. Vinod Prabhu[2], P. Kumar Gupta[3], K. Mukhopadhyay[1]

[1]Department of Biotechnology, Birla Institute of Technology, Mesra, Ranchi 835215, Jharkhand, India
[2]National Phytotron Facility, Indian Agriculture Research Institute, New Delhi 110012, India
[3]Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut 250004, India

Globally, wheat is the second most cultivated crop after rice and contributes 30% in total grains worldwide. The leaf rust disease of wheat poses a major threat, as it is prevalent worldwide and causes upto 40% crop loss under high severity. Different functional genomics approaches were used to study the wheat-leaf rust interaction but hexaploid large genome (17 Gb, AABBDD), polyploidy and high content of repetitive DNA slow down these studies. To overcome the challenges, SOLiD SAGE whole transcriptomic approach was used in the present study to effectively dissect and analyze this particular plant-pathogen interaction. Four SOLiD SAGE libraries were prepared for susceptible (HD2329) and resistant (HD2329 + *Lr28*) wheat plants, either mock inoculated or infected with the virulent pathogen *Puccinia triticina* pathotype 77-5. The libraries were quantified and purified using the highly sensitive Agilent 2100 Bioanalyzer and were sequenced on next-generation SOLiD (Applied Biosystems) sequencing platform generating more than 2Gb sequence data equivalent to 12x chromosome coverage per tag library. Comparative analysis of the data showed that expression of defense and stress responsive genes, transcription factors and pathogenesis-related proteins increased significantly during incompatible interaction in the resistant wheat plants.

Identification and location of chromosome-pairing promoters on chromosome 3B of bread wheat

Gay Georges[1]*, C. Rustenholz [1, 2]*, M.R. Perretant [1], E. Paux[1], M. Bernard[1], J. Able[3], J. Dvorak[4], G. Moore[5], C. Feuillet[1], P. Sourdille[1]

[1]INRA UMR Genetic, Diversity & Ecophysiology of the Cereals, 234 Av du Brézet, 63100 Clermont-Ferrand – France
[2]Iowa State University Ames, Iowa 50011-3650, USA
[3]The University of Adelaide, Waite Main Building, School of Agriculture, Food & Wine, South Australia 5064, Australia
[4]Department of Agronomy and Range Science, University of California, Davis, CA 95616, USA
[5]John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

**the two first authors contributed equally to this work*

Despite its polyploid nature and the close relationships between homoeologous chromosomes, bread wheat (*Triticum aestivum* L.) behaves like diploid species at meiosis and homoeologous chromosomes never pair between each other in normal euploid individuals, pairing being restricted to homologous chromosomes only. The genetic control of chromosome pairing in wheat is dependent on a series of suppressing and promoting pairing homoeologous (*Ph*) genes. Meiotic behavior of lines with abnormal chromosome stocks (aneuploid lines) suggests that several genes controlling homoeologous pairing exist and that the main factor that prevents homoeologous pairing (*Ph1*) is located on the long arm of chromosome 5B. It was also demonstrated that a gene located on the long arm of chromosome 3B that does not interact with *Ph1* is also necessary for normal pairing. In this work, we studied the variation of pairing behavior in the set of deletion lines from chromosome 3B. We showed that at least two genes located on the long arm of chromosome 3B have an influence on chromosome pairing in wheat. In a second step, we used the genes physically assigned to contigs on chromosome 3B to map genes preferentially expressed during meiosis in order to identify putative candidate genes implicated in pairing. Possible relation to the *Ph1* gene will be discussed.

Shift in expression of hormonal-related genes result in enhance drought tolerance in wild emmer wheat

T. Krugman[1], Z. Peleg[1]*, L. Quansah[2], A.B. Korol[1], E. Nevo[1], Y. Saranga[4], B. Chalhoub[3], A. Fait[2], T. Fahima[1]

[1]Department of Evolutionary and Environmental Biology, Institute of Evolution, Faculty of Natural Sciences, University of Haifa, Mt. Carmel, Haifa 31905, Israel
[2]Blaustein Institute for Desert Research, Department of Dryland Biotechnology, Ben Gurion University of the Negev, Midreshet Ben Gurion 84990, Israel
[3]Unité de Recherche en Génomique Végétale (URGV), Organisation and Evolution of Plant Genomes, 91057 Evry, France
[4]The Robert H. Smith Institute of Plant Science and Genetics in Agriculture, the Hebrew University of Jerusalem, Rehovot 76100, Israel
[*]Present address: Department of Plant Sciences, University of California, Davis, CA 95616, USA

Wheat production is severely affected in many growing areas of the world due to water deficiency. In the current study, transcriptome and metabolome profiles were used to unravel drought-adaptive mechanisms in roots and shoots of wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides)*, the progenitor of cultivated wheat. A comparative analysis was conducted between two wild emmer genotypes contrasting in their yield and yield stability under water-stress. The comparison between the drought resistant (R) and the drought susceptible (S) genotypes revealed significant differences in expression of genes involved in multilevel regulation processes, including transcriptional and post transcriptional regulation and ABA related genes, both in the flag leaves and in roots. Analysis of ABA content in the roots revealed that ABA content was higher in the well-watered treatment and further increased in response to drought in the R genotype, while in the S genotype ABA levels were constant. Furthermore, in the roots, significant changes were identified in expression of genes involved in biosynthesis, signalling and response of other phytohormones, mainly gibberellins and auxins. In addition to shift of hormone related genes, we have identified an induction of transcripts encoding RNA binding proteins, and various signalling proteins, including calcium (calmodulin, caleosin, and annexin), phosphatidylinositol, and WD-40 proteins. The metabolomic profiling revealed higher accumulation of intermediates of the tricarboxylic acid (TCA) cycle, which is central element of carbon metabolism in higher plants, both in the fully-watered and water-stressed roots of the R genotype. Higher accumulation of drought-related metabolites, involved in cell homeostasis, including glucose, trehalose, proline and glycine were also identified in the R genotype. The integration of transcriptomics and metabolomic results indicated that adaptation to drought in wild emmer wheat included efficient regulation and signalling pathways leading to effective bio-energetic processes, carbon metabolism, and cell homeostasis. The indentified genes serve as source for a thoughtful selection of potential candidate genes to improve drought tolerance in wheat, and further study are ongoing in our lab to characterize and use these genes.

Oral Presentations

Session 2
Mapping and Cloning

Towards the fine mapping and cloning the disease resistance genes from different sources in genome of wheat

X. Wang, Aizhong Cao, Bi Qin, Lei Xu, Liping Xing, Haiyan Wang, Tingting Chen, Chunmei Wang, Dajun Liu, Peidu Chen

State key Lab of Crop Genetics and Germplasm Enhancement, Cytogenetics Institute, Nanjing Agricultural University, Nanjing, Jiangsu210095, China

Various diseases are one of the main problems challenging wheat production. The identification of disease resistance genes from diverse resources and growing resistant varieties are the most effective and economical way to control diseases. In Cytogenetics Institute of Nanjing Agricultural University, we have focused on the identification, mapping and cloning of disease resistance genes from wheat as well as wheat relatives. The stripe rust resistance gene *Yr26*, which was introduced from *Triticum turgidum*, was mapped to the centromere region of chromosome 1B. Although the two B genomes of *T. turgidum* and common wheat have close relationship, the recombination suppression within this region makes it difficult to fine map the gene. The powdery mildew resistance gene *Pm6* was introduced from *T. timopheevii*. Although *Pm6* was mapped to the distal part of chromosome arm 2GL, due to the relatively further genetic distance of the genomes G and B, we also found low frequency of recombination near the *Pm6* locus. For the fine mapping of the *Yr26* and *Pm6* genes, we applied a comparative strategy to develop new markers near the target region. The *Pm21* gene was transferred from *Haynaldia villosa*, and was present in the wheat background as a 6VS/6AL translocation, *Pm21* was located on chromosome arm 6VS. The extremely low chance of recombination of V genome chromosomes and wheat chromosome makes the fine mapping and classical map-based cloning of *Pm21* rather difficult. To tackle these problems, we narrowed the chromosome region of *Pm21* by developing new alien chromosome lines involving 6VS, identify candidate genes by high throughout expression profile using the Affymetix GeneChip, validated the function by genetic transformation and VIGS, and confirm their target region location using the developed alien chromosome lines. We will present our research progresses and discuss the selection of appropriate strategies for mapping and cloning genes from different species and located in different chromosome regions.

Towards positional cloning of SV2, an specific adult plant wheat leaf rust resistance gene identified in the durable resistant variety Sinvalocho MA

M.J. Diéguez [1], M.F. Pergolesi[1], C. Petignat[1], M. Velásquez[1†], L. Ingala[1], M. López[1], M. Darino[1], F. Choulet[2], E. Paux[2], C. Feuillet[2], F. Sacco[1]

[1]Instituto de Genética "Ewald A. Favret" CICVyA-INTA CC25 (1712) Buenos Aires, Argentina
[2]UMR INRA-UBP 1095 Génétique, Diversité et Ecophysiologie des Céréales 234, Avenue du Brézet 63100 Clermont-Ferrand, France
[†]Present address: Laboratorio de Fisiología y Biología Molecular IFIByNE (CONICET) FCEyN-UBA, Intendente Güiraldes 2160 (C1428EGA) Buenos Aires, Argentina

The characterization of resistance genes, particularly from varieties that show durable resistance, may provide an outstanding contribution for controlling rusts in a context of sustainable agriculture. Leaf rust, caused by the biotrophic fungus *Puccinia triticina*, is one of the most important diseases of wheat worldwide, causing in Argentina annual yield losses of about 5-10%. *LrSV2*, a major gene identified in cv. Sinvalocho MA, confers adult plant resistance (APR) to leaf rust and was postulated as one of the determinants of the durable resistance observed in this cultivar, together with *LrSV1*, and probably other unidentified minor genes. Under field conditions, recombinant inbred lines (RILs) carrying *LrSV1* or *LrSV2* reduce the number of pustules by 40 and 35%, respectively, and 46% when they are both present. *LrSV1* was assigned to chromosome 2DS and is either an allele or closely linked to *Lr22* while *LrSV2* was mapped on sub-distal chromosome 3BS. It is located in the same region as the seedling leaf rust resistance gene *Lr27* whose expression is affected by the complementary gene complex *Lr12/31* on chromosome 4BL. In one of the F2 mapping populations used in this study, the specific *LrSV2* resistance was shown to be also affected by the segregation of a complementary gene complex on 4BL, suggesting that a similar mechanism regulates the expression of both genes. Using an F2 population of 3924 plants, *LrSV2* was flanked distally by marker cfp1410 and proximally by marker cfb5008, defining a physical interval of 262Kb on the 3B physical map of Chinese Spring. Sequence information from contigs in this interval revealed the presence of 4 tRNAs and 7 ORFs, including 4 pseudogenes. These 4 pseudogenes showed similarities to disease resistance RPM1-like protein, the beta-expansin 1a precursor and two tyrosine kinase domain containing proteins. Primers were designed that allowed the amplification of these sequences from genomic DNA of both Sinvalocho and Chinese Spring and experiments are underway to test their expression. In order to study this interval encompassed by markers cfp1410 and cfb5008 in Sinvalocho MA, a 3B chromosome BAC library from this cultivar will be constructed.

Low homology in the neighborhood of Resistance to *Puccinia hordei* QTL2

F.K.S. Yeo[1], B. Chalhoub[2], R.E. Niks[1], T.C. Marcel[3]

[1]Laboratory of Plant Breeding, Graduate school for Experimental Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands
[2]Unité de Recherches en Génomique Végétale (URGV-INRA), 2 rue Gaston Crémieux - CP 5708, 91057 Évry cedex, France
[3]INRA-UR 1290 BIOGER-CPP, Avenue Lucien Brétignières BP 01, F-78850 Thiverval-Grignon

Rphq2 is barley QTL for basal host resistance to *Puccinia hordei*. It confers a prolongation of *P. hordei* latency period of about 6% or 11 hours at seedling stage but much less so at the adult plant stage. It was identified in the recombinant inbred line populations L94 x Vada and Vada x SusPtrit, in a region with high recombination near the telomere of Chromosome 2HL. *Rphq2* was pinned into a genetic window of about 0.1 cM between markers WBE114 and WBE115. This interval displays synteny with a 61 Kb stretch of rice Chromosome 4 and with a 16 Kb stretch of *Brachypodium distachyon* contig Bd5. The gene underlying *Rphq2* could be a resistance factor in Vada, or a susceptibility factor in SusPtrit. Hence, we constructed two pooled BAC libraries, one each for Vada and SusPtrit. The Vada BAC library consists of ~ 161,000 clones with an average insert size of 82 Kbp per clone, corresponding to 3 genome-equivalents. The SusPtrit BAC library consists of 173,000 clones with an average insert size of 118 Kbp per clone, corresponding to 4 genome-equivalents. A contig of three BAC clones from Vada spanning the QTL region was successfully constructed. The region between flanking markers is a stretch of ~191 Kb. For SusPtrit, only a partial contig was obtained using 3 BAC clones. The left end of the partial contig consists of one BAC with the size of ~100 Kb and the right end has two overlapping BAC clones which together gives ~199 Kb. The homology of the two orthologous sequences is low. Indeed, while some genes are common between Vada and SusPtrit, others are unique to Vada or SusPtrit.

Chromosome genomics facilitates cloning of a Russian wheat aphid resistance gene

H. Simkova[1], H. Stankova[1], M. Valarik[1], J. Safar[1], N. Lapitan[2], M.-C. Luo[3], S. Taudien[4], N. Stein[5], K.F.X. Mayer[6], P. Berkman[7], D. Edwards[7], J. Dolezel[1]

[1]Centre of the Region Hana, Institute of Experimental Botany, Sokolovska 6, CZ-77200 Olomouc, Czech Republic
[2]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, Colorado, 80524, USA
[3]Department of Plant Sciences, University of California, Davis, CA 95616, USA
[4]Fritz Lipmann Institute, Beutenbergstrasse 11, D-07745 Jena, Germany
[5]Leibniz Institute of Plant Genetics and Crop Plant Research, Department Genebank, AG Genome Diversity, D-06466 Gatersleben, Germany
[6]MIPS/IBIS, Helmholtz Zentrum München, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany
[7]Australian Centre for Plant Functional Genomics, University of Queensland, Brisbane, QLD 4072, Australia

Positional cloning in bread wheat (*Triticum aestivum* L.) is hampered by the enormous genome size (~ 17 Gbp), polyploidy, and prevalence of repetitive DNA. These obstacles can be largely overcome by dissecting the genome into particular chromosome arms by flow-cytometric sorting. This strategy enables construction of subgenomic BAC libraries, physical maps as well as gaining shotgun sequences of particular chromosome arms, which are favorable resources for cloning genes in the hexaploid wheat. With the aim to clone a Russian wheat aphid resistance gene *Dn2401* located on the short arm of chromosome 7D (7DS), we have constructed a 7DS-specific BAC library representing 12.1 arm equivalents. The library has been fingerprinted and BAC contigs have been assembled. The library was PCR-screened with *Dn2401*-linked microsatellite markers delimiting an interval of 2.5 cM and BAC contigs comprising these markers were identified. Two BACs from each contig were completely sequenced by 454, which revealed genes flanking the region of interest. Using the GenomeZipper created by combining 454 reads of particular barley chromosomes, barley ESTs and genome sequences of Brachypodium, rice and sorghum, we succeeded in delimiting syntenic regions in these genomes. The GenomeZipper data was projected on sequence assembly obtained from paired-end Illumina sequencing of 7DS arm and relevant wheat region was extracted. The 7DS sequence assembly comprises majority of 7DS genes as well as gene-flanking regions, which makes it possible to design primers for extragenic regions showing higher level of polymorphism. Availability of sequence assemblies for all homoelogous group 7 short arms (www.wheatgenome.info) facilitated primer design amplifying specifically 7DS sequences. This approach supported targeted development of new SNP markers from the region of interest and shows to be a promising strategy to cope with the low polymorphism of wheat D genome.

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Positional cloning and analysis of genes coding for fructan biosynthesizing enzymes in wheat and barley

B.L. Huynh[1, 2], D. Mather[3], Z. Shoaei[1], A. Schreiber[1], J. Toubia[1], N. Stein[4], J. Stangoulis[5], P. Langridge[1], D. Fleury[1]

[1]Australian Centre for Plant Functional Genomics, PMB 1, Glen Osmond, SA 5064, Australia
[2]Phenomics and Bioinformatics Research Centre, School of Mathematics and Statistics, University of South Australia, Mawson Lakes, SA 5095, Australia
[3]School of Agriculture, Food and Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia
[4]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany
[5]School of Biological Sciences, Flinders University, GPO Box 2100 SA 5001, Australia

Fructans are polymers of fructose molecules, which confer health benefits by acting as prebiotics that selectively stimulate the growth of bifidobacteria in the digestive system. Fructans are present in the grain and vegetative organs of temperate cereals such as wheat and barley. A major QTL controlling grain fructan concentration in wheat has been mapped on chromosome 7A. By mapping the gene encoding sucrose:sucrose 1-fructosyltransferase (1-SST), a pacemaker enzyme involved in fructan synthesis, we found that the 1-SST gene coincided with the 7A fructan QTL. More candidate genes were then mapped using a barley-wheat comparative mapping approach. By screening DNA pools from barley BAC libraries (cv. Morex), the 1-SST gene was anchored onto a barley BAC physical contig. Two BAC clones flanking the gene were then sequenced. The 1-SST gene clustered together with two other fructan-biosynthetic genes coding for fructan-fructan 1-fructosyltransferase (1-FFT) and sucrose: fructan 6-fructosyltransferase (6-SFT) in addition to multiple copies of the vascuolar invertase (VIN) gene and transposable elements. We hypothesized that fructan biosynthetic genes might have evolved from their ancestor gene, VIN, through gene duplication caused by transposon activity. Gene-based SNPs for 1-FFT, 6-SFT and VIN were developed and successfully mapped to the fructan QTL. These markers were co-dominant and enabled differentiation of high versus low-fructan cultivars and breeding lines. They could therefore be used in molecular breeding for high fructan wheat.

Map based cloning and characterization of Ug99 resistance gene *Sr35*

C. Saintenac[1], W. Zhang[2], M. Rouse[3], E. Akhunov[1], J. Dubcovsky[2]

[1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66502, USA
[2]Department of Plant Sciences, University of California, Davis, CA 95616, USA
[3]USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108, USA

Puccinia graminis f. sp. *tritici*, the causal agent of wheat stem rust, recently received a great deal of attention due to the emergence of a new devastating race called Ug99. Discovered in east Africa, it poses a serious threat to the world wheat production. To face this challenge, many genes that confer resistance to Ug99 have been identified and are actively being used for developing new wheat varieties. Among them, the *Sr35* resistance gene discovered in *T. monoccocum* also shows resistance to Ug99 and all Ug99-derived races of stem rust. We previously mapped *Sr35* to the distal part of the long arm of chromosome 3A using different diploid and hexaploid wheat populations. By taking advantage of conserved synteny between wheat and *Brachypodium* in this region and using a large F2 mapping population, we mapped *Sr35* to a 0.1 cM interval. The sequencing of BAC clones spanning this interval in two resistant *T. monoccocum* accessions resulted in 300 kb contig harboring a cluster of closely related NBS-LRR genes and pseudo-genes. The analysis of gene structure and expression was used to reduce the number of candidates to three. Forward genetic screening of an EMS mutant population derived from the resistant *T. monoccocum* accession G2919 allowed for the discovery of a susceptible M2 family. The sequencing of candidate genes in this mutant discovered the presence of a premature stop codon in one of the three NBS-LRR genes. The association of the identified candidate gene with resistance phenotype was further confirmed in a panel of resistant (*Sr35*+) and susceptible (*Sr35*-) *T. monococcum* accessions. The functional validation of this gene by VIGS and transgenic complementation is currently underway.

Using sequence of flow-sorted chromosome 3B to develop SNP and InDel markers for genetic mapping

M. Shatalina[1], T. Wicker[1], J. Dolezel[2], H. Simkova[2], B. Keller[1]

[1]Institute of Plant Biology University of Zürich Zollikerstrasse 107 8008 Zürich Switzerland
[2]Institute of Experimental Botany Sokolovska 6 CZ-77200 Olomouc Czech Republic

Bread wheat is a hexaploid and has a highly repetitive large genome. Therefore, construction of a high-resolution genetic map for a particular wheat population is a complex task. The most reliable and reproducible markers are sequence-based, but since the wheat genome is not sequenced at the moment, the other possible option is to develop markers from sequence fragments. Our mapping population is derived from a cross of Swiss winter cultivar Arina and Swiss winter cultivar Forno. In order to enrich its genetic map with markers, 3B chromosomes of two parental cultivars were flow-sorted and shotgun sequenced using Illumina technology. The obtained sequence data gave a total coverage of nine fold per sample. We used synteny between wheat and *Brachypodium distachyon* to select sequences of potential interest. Selected contigs containing single nucleotide polymorphisms and insertions or deletions are being used to produce new markers for the genetic map.

Oral Presentations

Session 3

How to bridge potential and practice? Applied Molecular Breeding

Molecular breeding in cereals: current status and perspectives

V. Korzun

KWS LOCHOW GMBH, 37574 Einbeck Germany
E-mail: viktor.korzun@kws-lochow.de

At the beginning of the 21st Century, mankind faces the challenge of feeding a growing population with increasing demands in the quality of food. Forecasts of climate change indicate additional uncertainty and place even more pressure on our natural resources to provide enough food for everyone. In this context, genomics applied to agriculture and more specifically to cereal production must play an important role in sustainably balancing the world's food supply and demand by developing new varieties that are adapted to the different environmental conditions and make better use of inputs. Conventional cereal breeding is time-consuming and depends on environmental conditions. Therefore, breeders are extremely interested in new technologies that could make this procedure more efficient. Molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improve selection strategies in breeding. There has been a large and rapid accumulation of genomics tools in cereal crops during last decade. These developments have been coupled with the emergence of high throughput technologies, which have allowed advances in molecular marker technology and implementation. The complete restoration of pollen fertility and reduced infection by the ergot fungus in breeding hybrid varieties in rye and resistances in wheat and barley are prime targets in practical breeding. In all cases the breeding process can be accelerated by applying molecular markers for developing new material and for improvement of the selection intensiveness and accuracy. The detailed results of such specific applications of molecular markers in cereals, association mapping and potential of genomic selection will be presented and discussed.

Predicting Wheat Yield with Markers

E. Storlie, G. Charmet

INRA, UMR GDEC, 234 ave du brezet, 63100, Clermont-Ferrand, France

Prediction of trait values via genomic selection (GS) has evolved from theory to implementation during the last decade, notably in dairy cattle. Breeders of most animals and plants can imagine some benefits of GS – reduced generation and phenotyping time and space, for example. Linkage disequilibrium between markers and QTLs may be a hurdle for some crops, like wheat, which are currently constrained by relatively sparse molecular maps. Despite the sparsity, estimation of Breeding Values (EBV) may have predictive value (Zhang et al., 2011) and may lend insight into the utility of GS for improving wheat. Our investigation analyzed yield data of 318 elite wheat lines, genotyped with 2236 DArT markers, and grown at 7 locations during a ten year period in France. They were analyzed for their abilities to predict the yield of genotypes using four models – Pedigree BLUP, Ridge Regression BLUP, Bayesian Ridge Regression and Lasso. We addressed the question of marker density and predictability, after randomly eliminating markers and re-estimating correlations between estimated and true breeding values. We also tested predictability of the models using year-specific or location-specific target populations; differences with random target populations may suggest a GxE effect.

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Genomic Prediction of Breeding Traits in Bread Wheat

Y. Manès, J. Crossa, S. Dreisigacker

International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico DF, Mexico.

Traditional marker-assisted selection (MAS) strategies relying on QTL detection have not greatly accelerated improvement of the highly polygenic quantitative traits such as yield. Genomic selection (GS) is a new approach that couples the phenotypic data generated routinely by plant breeders and high density marker information. It relies on recently developed statistical approaches that use the marker information as a whole to improve prediction of phenotypic performance, rather than focusing on detecting the most significant genetic effects like in QTL mapping. CIMMYT has tested diverse GS models on grain yield, rust and heading date data generated on 306 elite lines. Grain yield data from two environments in 2009 and five in 2010 in C.E.N.E.B (Campo Experimental Norman Ernest Borlaug, Cd Obregón, Sonora, North of Mexico), stem rust Ug99 notes from three seasons of scoring in Kenya, and heading date data from five environments scored in 2009 and 2010 in Mexico were included in the models. Lines were genotyped with 1667 DArT polymorphic markers. Correlations between the predictive and the observed values were computed using a 10-fold cross-validation scheme that predicts 10% of the missing lines. Models tested were the standard infinitesimal model relying on pedigree information (P), the Bayesian lasso (BL) and the Bayesian reproducing Kernel Hilbert Spaces (RKHS) models with markers alone, and the two latter combined with pedigree information (PBL and PRKHS, respectively). Considering averages of all environments, the best correlations were obtained with the PBL models, with values of 0.78, 0.71 and 0.85 for heading date, stem rust and yield respectively. Correlations using only marker information were also high for the three traits, but slightly lower than when marker and pedigree information were combined in the model. Considering average of all environments, predictions with BL were 0.76, 0.67 and 0.82 for heading date, stem rust and yield. Examining individual environments, correlation increases with heritability in the case of grain yield. This underscores the importance of good quality data for the implementation of GS in plant breeding programs. These preliminary results are promising, especially considering the relatively low number of lines and markers in this data set. Next step will be the application of GS as early as F2 in wheat breeding schemes, selecting and intercrossing F2 plants purely on the basis of genetic information. This should accelerate the accumulation of favorable alleles and therefore increase genetic gains per time.

Marker assisted breeding for improving Fusarium head blight resistance in wheat

H. Buerstmayr[1], A. Salameh,[1,2], M. Buerstmayr[1], B. Steiner[1], A. Neumayer[3], M. Lemmens[1]

[1]BOKU - University of Natural Resources and Applied Life Sciences Vienna, Department IFA-Tulln, Institute for Biotechnology in Plant Production, Konrad Lorenz Str. 20, A-3430 Tulln, Austria
[2]current address: Hebron University, Palestine
[3]Saatzucht Donau GmbH & Co KG, breeding station Reichersberg, A-4981 Reichersberg am Inn, Austria

Breeding for Fusarium head blight (FHB) resistance of wheat is a continuous challenge for plant breeders. Resistance to FHB is quantitative trait, governed by several to many genes and modulated by environmental conditions. QTL for FHB resistance have been detected on virtually all wheat chromosomes, but only a few have been validated so far for their usefulness in breeding (Buerstmayr et al. 2009). The presented study was undertaken to assess the effect on improving FHB resistance of two resistance QTL, namely *Fhb1* and *Qfhs.ifa-5A*, from the CIMMYT spring wheat line CM-82036 when transferred by marker assisted back-crossing in European winter wheat. To achieve these goals we developed and evaluated fifteen back-cross-two derived families based on nine European winter wheat varieties as recipients and the FHB resistant line CM-82036 as resistance donor. Winter wheat back-cross lines with one QTL from the resistant donor showed a clear tendency towards increased FHB resistance. On average lines with *Fhb1* plus *Qfhs.ifa-5A* combined were only slightly more resistant compared to lines with *Fhb1* alone. The obtained results suggest that the effect of the spring wheat derived QTL on improving FHB resistance increases in the order *Qfhs.ifa-5A* < *Fhb1* <= *Qfhs.ifa-5A* plus *Fhb1* combined. The genetic background of the recipient line had huge impact on the resistance level of the obtained lines. No systematic negative effect of the spring wheat derived QTL on grain yield, thousand grain weight, hectolitre weight and protein content was found. The use of spring wheat derived FHB resistance QTL for breeding high yielding cultivars with improved FHB resistance appears therefore highly promising. For more details see Salameh et al. (2010).

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Using a Multi-parent genetic population to generate a high resolution SNP genetic map and exploring the underlying genetic architecture

C. Cavanagh[1], E. Huang[1], M. Hayden[2], S. Chao, E. Akhunov[3], A. George[1], M. Morell[1]

[1]CSIRO, Food Futures National Research Flagship, Canberra, ACT 2601, Australia
[2]Department of Primary Industries Victoria, Victorian AgriBiosciences Center, 1 Park Drive, Bundoora, VIC 3083, Australia
[3]USDA-ARS Biosciences Research Laboratory, Fargo, ND, USA
[4]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA

The complexity of the wheat genome, due to polyploidy and the high level of repetitive sequences, along with the low level of polymorphism in elite wheat cultivars has meant that generating high resolution genetic maps from representative cultivars has been a challenging endeavour. Here, we present a genetic map in wheat developed from a 4-parent multi-parent advanced generation intercross (MAGIC) populatuion. In collaboration with several research groups and the International Wheat SNP Working Group we have utilised a 9k infinium assay to genotype SNPs and in addition we have included DArT and SSR markers. We will provide a summary of the results and compare with other maps available. One complicating factor in establishing genetic maps in wheat has been the difficulties associated with adequately dealing with alien translocations and including them in the genetic map. Here, we discuss how we have used the MAGIC population to include a well known alien translocation into the genetic map. In order to exploit genomic selection or genome wide association an understanding of the level of linkage disequilibrium (LD) across the genome is essential in determining the number of informative markers that are required for successful implementation. We examine the extent of LD within the MAGIC population and discuss the implications of these findings on breeding.

Molecular breeding using transgenic wheat: a case study for fungal disease resistance

B. Keller, G. Herren, S. Brunner

University of Zürich, Institute of Plant Biology, Zollikerstrasse 107, 8008 Zürich Switzerland

Because of mostly political arguments, field research with transgenic wheat has been slow in the last decade. However, in the long-term genetically modified wheat might represent a highly relevant new approach to improve agronomically important traits. In our group, we have worked specifically on transgenic fungal disease resistance. In wheat (*Triticum aestivum* L.), the highest yield losses due to pathogens worldwide are caused by fungal diseases. Among them is powdery mildew caused by *Blumeria graminis* f. sp. *tritici*, which attacks epidermal cells of leaves. We are studying the molecular diversity and function of the wheat powdery mildew resistance (R) gene Pm3. It has 17 functional alleles (*Pm3a-g* and *Pm3k-t*) that confer race-specific resistance and encode members of the large class of CC-NB-ARC-LRR proteins. For the Pm3a-d, Pm3f and Pm3g alleles, we have developed transgenic lines expressing a single, epitope-tagged PM3 derivative driven by the constitutive, strong ubiquitin promoter. Powdery mildew resistance was significantly improved in all lines in the greenhouse and the field, both with naturally occurring infection or after artificial inoculation. Under controlled environmental conditions, the line with the strongest overexpression of the Pm3b gene showed a dramatic increase in resistance to several independent isolates that are virulent on lines carrying an endogenous copy of Pm3b. In one or more field environments, but never in the greenhouse, five of the 12 tested transgenic lines showed pleiotropic effects on leaves and/or spikes. The strongest overexpressing line had the most dramatic negative effects, suggesting a correlation between the expression level and phenotypic changes. These results demonstrate that the successful transgenic use of R genes will critically depend on achieving an optimal level of their expression, possibly in a tissue specific way. Since single major R genes may be rapidly overcome in lines with widespread cultivation, we tested if mixtures of transgenic lines carrying each a different Pm3 allele represent a long term agronomical strategy to increase R gene durability. We will also describe some of the political and legal questions that were raised and sometimes answered by this field experiment.

Characterization of a wheat transcription factor, *TaWRKY45*, and its overexpression confers multiple fungal disease resistances in transgenic wheat plants

I. Bahrini[1], T. Ogawa[2], H. Kawahigashi[2], M. Sugisawa[3], R. Kikuchi[3], F. Kobayashi[2], T. Ban[3], H. Handa[1, 2]

[1]Graduate School of Life and Environmental Sciences, University of Tsukuba, 305-8572 Tsukuba Japan
[2]Plant Genome Research Unit, National Institute of Agrobiological Sciences, 305-8602 Tsukuba Japan
[3]Kihara Institute for Biological Research, Yokohama City University, Yokohama 244-0813, Japan

The WRKY transcription factors belong to a large protein family characterized by the conserved WRKY domain. These factors have been identified to play biological functions in various plant developmental processes. WRKY proteins are also known to be involved in regulating plant responses to pathogen attacks and stress-related hormones. In this study, we report the isolation and characterization of the gene (*TaWRKY45*) for the wheat WRKY45 transcription factor. Amino acid sequence alignment and phylogenetic analyses demonstrated that the TaWRKY45 protein is orthologous to rice OsWRKY45. Our analysis of its expression in wheat indicated that *TaWRKY45* was constitutively expressed in various organs and throughout the lifetime of the plant. We observed that *TaWRKY45* was upregulated in response to benzothiadiazole (BTH), a plant immune system strengthener, and also in response to the infections with three fungal pathogens; *Blumeria graminis*, a causal fungus for powdery mildew, *Fusarium graminearum*, a causal fungus for *Fusarium* head blight (FHB) and *Puccinia triticina*, a causal fungus for leaf rust. The constitutive overexpression of the *TaWRKY45* transgene conferred enhanced resistances against these three fungi to transgenic wheat plants grown under greenhouse conditions. However, the expressions of two resistance-related genes, *Pm3* and *Lr34*, were not induced in *TaWRKY45*-overexpressed wheat plants by the inoculation with powdery mildew fungus. These results suggest that *TaWRKY45* is involved in the defense systems for the multiple fungal diseases in wheat but *TaWRKY45*-involving resistance is different from at least *Pm3* and/or *Lr34*-related resistances. Our present study indicates that *TaWRKY45* is involved in the defense systems for the biotic stressors in wheat and that it may be potentially utilized to improve the wide range of disease resistances in wheat.

Oral Presentations

Session 4

Exploring and exploiting Triticeae Genetic Resources

National BioResource Project-Wheat, Japan: Conservation and Utilization of Genetic Resources of Wheat

S. Nasuda[1], M. Nitta[1], T. Kawahara[1], T. R. Endo[1], M. Kishii[2], T. Ban[2], K. Kawaura[2], Y. Ogihara[2]

[1]Laboratories of Plant Genetics, and of Crop Evolution, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
[2]Kihara Institute for Biological Research, Yokohama City University, Yokohama 244-0813, Japan

In 2002, the Japanese government launched the National BioResource Project (NBRP) in order to promote scientific researches collectively not only in Japan but also in the world. The aims of NBRP are the strategic propagation, maintenance, and distribution of living organisms, cells and DNA clones of major plant and animal species, one of which is wheat. NBRP-Wheat (Komugi in Japanese) is currently carried out by the Graduate School of Agriculture, Kyoto University (core facility) and the Kihara Institute for Biological Research, Yokohama City University (sub-facility). During the first stage of NBRP-Wheat, 2002-2006, more than 7000 wheat stocks, including wild species, landraces and genetic stocks, have been collected, propagated and conserved. Also, more than a million genomic DNA and EST clones have been collected and conserved. All data is stored in the database KOMUGI (http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp). Since 2007, the second stage of NBRP-Wheat has started to achieve one of the best collections of wheat genetic stocks in the world. The NBRP-Wheat project stores and supplies wild species, landraces, and experimental strains of wheat and related species (currently providing ca. 12000 accessions). The wheat researches can access to the genome resources of 570000 cDNA sequences and TAC clones through the KOMUGI database. In the second stage, in addition to the our primary task of maintenance and distribution of the genetic and genomic resources, we are focusing on surveying polymorphisms among wheat accessions that would be useful in genetic studies and wheat breeding. The objectives of the DNA marker project are, (1) to make the resources of the NBRP-Wheat more valuable for molecular studies by addition of genotype information, and (2) to find a set of DNA markers that is suitable for detecting polymorphisms among hexaploid wheat accessions. We have surveyed amplification profiles of the publically available 1600 SSR markers in 48 accessions of cultivars of hexaploid wheat and their relatives. Through the profiling we selected a set of 210 SSR markers that is suitable for surveying polymorphisms of hexaploid wheat. We started to study phenotypic and genotypic variation of our hexaploid wheat accessions in order to establish a core-collection of our stocks.

Comparative analysis of D-genome diversity in *Aegilops tauschii*, common bread wheat (*Triticum aestivum*) & synthetic hexaploid wheat

N. Gosman[1]*, H. Jones[1]*, R. Horsnell[1], A. Kowalski[1], G. Rose[1], L. Everest[1], A. Bentley[1], S. Tha[2], C. Uauy[2], D. Novoselovic[3], R. Simek[3], B. Kobiljski[4], A. Kondic-Spika[4], O. Mitrofanova[5], Y. Chesnokov[5], A. Greenland[1]

[1]National Institute of Agricultural Botany, Cambridge
[2]John Innes Centre, Norwich
[3]Agricultural Institute of Osijek
[4]Small Grains Breeding & Biotechnology Institute, Novi Sad
[5]Vavilov Institute, St Petersburg

* These authors contributed equally to the work

Wheat is an allohexaploid, combining within a single genome those of three diploid grass species, the A genome of *Trititcum urartu*, the B genome from a species related to *Aegilops speltoides* and the D genome of *Aegilops tauschii*. The allopolyploid origin of bread wheat undoubtedly contributes to its adaptability since its progenitors grow in a wide range of environments. *Aegilops tauschii* has a range from the southern shores of the Caspian Sea, across northern Iran, Turkmenistan and northern Afghanistan to China and may be expected to harbour adaptive diversity that would contribute to its fitness in differing environments. However, it is likely that only a few strains of *Ae. tauschii* contributed to the evolution of bread wheat resulting in a genetic 'bottleneck' reducing genetic variation in wheat overall and on the D genome in particular. The aims of our study were threefold, (1) genetic distance analysis to assess the D genome diversity currently available in commercial European bread wheat germplasm relative to what is available in *Ae. tauschii* from across its diversity range, (2) identify new sources of *Ae. tauschii* diversity that have not already been exploited by the CIMMYT wheat re-synthesis program, (3) project *Ae. tauschii* collection site data onto eco-geographic maps to identify accessions that, by implication, might carry traits of potential interest, e.g. drought, heat or cold tolerance. Three pools of D-genome diversity were genotyped with 15 SSR markers, 190 European bread wheat varieties from UK, France, Germany, Serbia and Croatia, 50 synthetic hexaploid wheat (SHW) lines developed at CIMMYT and 268 *Ae. tauschii* accessions from across the species range from Turkey, the trans-Caucus and Iran in the South and West to Kazakhstan and Pakistan in the North and East. As expected, average D-genome gene diversity in bread wheat was consistently lower than that of *Ae. tauschii*. Genetic distance analysis revealed a marked division of *Ae. tauschii* accessions into two groups. A mixture of *ssp tauschii* and *ssp strangulata* from the trans-Caucus, and countries bordering the Caspian Sea formed one group (A) and mostly *ssp tauschii* from counties to the north and east formed the other (B). The European bread wheat sample occupied a separate clade that was most closely associated with group A. Although no collection site data were available for accessions used to develop the CIMMYT synthetic wheat, the study sample was almost entirely incorporated within group A. Allele prevalence at the *Ppd-D1* flowering locus mirrored the pattern identified by genetic distance analysis with group A possessing a mixture of 411bp (bread wheat wt allele), 427bp and 450bp alleles, whilst the 450bp allele was fixed in group B. The adaptive significance, if any, of this

observation is unknown; however, a study is in progress at NIAB to identify whether the 427 or 450bp alleles affect flowering response. On the basis of our study and those of others, European bread wheat captures only a fraction of D-genome variation available in the *Ae. tauschii* gene pool. The CIMMYT SHW programme has apparently captured a relatively small, but highly diverse sample of additional diversity from around the trans-Caucus and trans-Caucasian Iran. For many years, the CIMMYT SHW programme has been a major source of additional D-genome diversity available to wheat breeders world-wide both via primary synthetics and synthetic-derived varieties. Recent funding from the BBSRC for a new re-synthesis programme at NIAB will incorporate a wider pool of novel D-genome diversity that will be available to the global wheat breeding community.

Lr34 multi-pathogen resistance ABC transporter: molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species

S.G. Krattinger[2], E.S Lagudah[2], T. Wicker[1], J.M Risk[2], A.R Ashton[2], L.L Selter[1], T. Matsumoto[3], B. Keller[1]

[1]Institute of Plant Biology, University of Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland
[2]CSIRO Plant Industry, GPO Box 1600, Canberra, ACT, 2601, Australia
[3]Plant Genome Research Unit, Division of Genome and Biodiversity Research, National Institute of Agrobiological Sciences, 2-1-2, Kannondai, Tsukuba, Ibaraki 305-8602, Japan

The wheat disease resistance gene *Lr34* confers durable, race non-specific protection against three fungal pathogens and has been a highly relevant gene for wheat breeding since the green revoluton. *Lr34*, located on chromosome 7D, encodes an ATP-binding cassette (ABC) transporter of 1,401 amino acids. Both wheat cultivars with and without *Lr34*-based resistance encode a putatively functional protein that differ by only two amino acid polymorphisms. In this study we focused on the identification and characterization of homoeologous and orthologous *Lr34* genes in hexaploid wheat and other grasses. In hexaploid wheat we found an expressed and putatively functional *Lr34* homoeolog located on chromosome 4A, designated *Lr34-B*. Another homoeologous *Lr34* copy, located on chromosome 7A, was disrupted by insertion of repetitive elements. Protein sequences of LR34-B and LR34 were 97% identical. In 16 wheat cultivars and 77 Chinese landraces, LR34-B showed the susceptible haplotype for the two critical polymorphisms distinguishing the LR34 proteins from susceptible and resistant wheat cultivars. Orthologous *Lr34* genes were detected in the genome sequences of rice and sorghum. Maize, Brachypodium, rye and barley were lacking *Lr34* orthologs, indicating independent deletion of this particular ABC transporter. Similar to LR34-B, the orthologs from rice and sorghum have the susceptible haplotype. Furthermore, in 250 accessions of *Aegilops tauschii*, the donor of the wheat D-genome, we exclusively detected the susceptible *Lr34* haplotype. We therefore conclude that the particular *Lr34*-haplotype found in resistant wheat cultivars is unique and evolutionary very young. The durable *Lr34*-resistance is most likely the result of gene diversification after hybridization of hexaploid wheat. Thus, human selection may have played an important role in fixing this unique haplotype in wheat germplasm.

Advanced back-cross QTL mapping of resistance to Fusarium head blight derived from *Triticum macha* (Georgian spelt wheat)

M. Buerstmayr, M. Lemmens, B. Steiner, H. Buerstmayr

BOKU-University of Natural Resources and Life Sciences Vienna, Department IFA-Tulln, Institute for Biotechnology in Plant Production, Konrad Lorenz Str. 20, A-3430 Tulln, Austria

While many reports on genetic analysis of Fusarium resistance in bread wheat have been published, only limited information is available on FHB resistance derived from wheat relatives. We report about genetic analysis of FHB resistance derived from *Triticum macha* (Georgian spelt wheat). As the origin of *T. macha* is in the Caucasian region it is supposed, that its FHB resistance differs from other resistance sources. In order to introduce valuable alleles from the landrace *T. macha* into a modern genetic background we used an advanced back-cross QTL mapping scheme (Tanksley and Nelson 1996). A back-cross-two derived recombinant inbred line population of over 300 BC_2F_3 lines was developed from a cross of *T. macha* with the Austrian winter wheat cultivar Furore. The population was evaluated for Fusarium resistance in six field experiments. The population was genetically fingerprinted using SSR and AFLP markers. Map construction was done with an updated version of *CarthaGène* (De Givry et al. 2005). For QTL mapping *QGene* (Nelson 1997) was used. The obtained linkage map covered 37 linkage groups with 563 markers. Five novel FHB resistance QTL, all descending from *T. macha*, were found on four chromosomes (2A, 2B, 5A, 5B). The largest effect QTL overlapped with the *Q-locus* (spelt type) on chromosome 5A and appears therefore an interesting QTL especially for spelt wheat improvement. For details see Buerstmayr et al. (2011).

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The application of molecular markers for wheat improvement: Breeding for stem rust resistance in Kazakhstan

A. Kokhmetova[1], F. Ogbonnaya[2], M. Atishova[1], G. Yesenbekova[1], A. Morgounov[3]

[1]Institute of Plant Biology and Biotechnology, Timiryazev st.45, Almaty, 050040, Kazakhstan
[2]International Centre for Agricultural Research in the Dry Areas, PO Box 5466, Aleppo, Syria
[3]CIMMYT-Turkey, P.K. 39 Emek 06511 Ankara, Turkey

Wheat rusts are important problems in Kazakhstan and one of the major factors reducing the productivity of this crop. Of the three rusts, stem rust of wheat is one of the most damaging diseases of wheat throughout the world. Recently, a virulent race of stem *(Puccinia graminis Pers.f.sp.tritici)* designated Ug99, "TTKS", resulted in the break down of stem rust resistance gene, *Sr31* which is widely deployed and found in most wheat varieties grown around the world. Since then, TTKS variants with virulence to other stem rust genes including *Sr24*, *Sr36*, etc has been reported posing serious threat to wheat production worldwide. Unlike other rusts, which only partially affect wheat yields, stem rust can cause up to 100% crop loss. Up to 80 per cent of all Asian and African wheat varieties are susceptible to the fungus and major wheat-producing nations to Iran's east – such as Afghanistan, India, Pakistan, Turkmenistan, Uzbekistan and Kazakhstan – should be on high alert FAO (2008) warned. Host resistance remains one of the most cost effective control measures. Thus knowledge of the genes present in current elite cultivars is imperative if effective breeding strategies are to be employed in combating the potential losses associated with break down in stem rust resistance. The aim of the present study was to screen elite advanced wheat lines from Kazakhstan with markers linked to stem rust genes *Sr22*, *Sr24*, *Sr25*, *Sr26*, and *Sr39* including the adult plant yellow rust resistance gene *Yr18*/*Lr34*. We also evaluated a wide range of wheat lines from various parts of the world against number races of *Puccinia graminis Pers.* collected in different part of Kazakhstan. Eight out of 42 genotypes possess *Sr22* and displayed DNA fragment associated with *Sr22* resistant gene. None of the lines possess *Sr25*, *Sr26* and *Sr39*. However, 7 lines displayed DNA-fragment associated with *Sr24* resistance when amplified with STS primer Sr24#12. A high number of the lines, 25 out of 42 possess yellow rust resistance gene Yr18/Lr34. Four of the lines have combined *Sr24* and *Yr18* stem and yellow rust resistance genes. These results would assist breeders in choosing parents for crossing in developing varieties with desirable levels of stem rust resistance in Kazakhstan.

SNP and haplotype identification in a large set of wheat genes

M. Ganal[1], A. Polley[1], E.M. Graner[1], R.Wiseseke[1], C. Huettl[1], H. Luerssen[1], G. Durstewitz[1], J. Plieske[1]

[1]TraitGenetics GmbH, Am Schwabeplan 1b, 06466 Gatersleben, Germany

SNP (single nucleotide polymorphism) markers are becoming increasingly important for genetic mapping and breeding purposes. However, random SNP markers are less informative than markers that are diagnostic for specific haplotypes. In order to investigate the SNP and haplotype structure of European elite wheat germplasm, we have developed primers for the amplification of a large number of wheat genes based on ESTs and genomic sequences. Altogether, we have analyzed nearly 20000 wheat genes through amplification of specific fragments in a set of 16 wheat lines of which 13 were European elite varieties. Sequencing was performed using the Illumina sequencing technology. Based on the sequencing data, we have identified SNPs and haplotypes in many of these genes. Optimization of SNP prediction and validation of identified SNPs was performed with a set of small genotyping arrays. The results of this study will permit the development of genotyping arrays based on haplotype-specific SNPs resulting in a set of highly polymorphic markers that will be very useful for wheat breeding.

Refining software tools for calling genotypes using high density SNP panels (>10,000 markers) in polyploid species

M. Hansen[1], E. Akhunov[2], T. Davis[3], J. de Haas[4], J. Dvorak[5], M. Hayden[6] J. Jacobs[7], M.C. Luo[5], A. Van Deynze[5], T. Royce[1], R. Hodgson[1], J. Peart[1], R. Yip[1], C. Lawley[1]

[1]Illumina, Inc. 9884 Towne Centre Drive, San Diego, CA 92121
[2]Kansas State University 4024 Throckmorton Plant Sciences Center, Manhattan, KS 66502 USA
[3]University of New Hampshire Department of Biological Sciences, NH 03824 USA
[4]HZPC, Roptawei 4, Metslawier, 9123JB Netherlands
[5]University of California, Department of Plant Sciences, Davis, CA 95616 USA
[6]Department of Primary Industries Victoria, Victorian AgroBiosciences Centre, 1 Park Drive, Bundoora 3083, Australia
[7]Plant and Food Research, Private Bag 4704, Christchurch 8140 New Zealand

The ability to collect vast amounts of high quality sequencing data for low cost has facilitated assembly and SNP discovery in many complex genomes. Simultaneous with throughput advances in sequencing, are lower costs and higher flexibility in SNP genotyping technology. The target application for low, mid and high-plex SNP panels in many plants include research, validation and screening of high numbers of lines for breeding purposes. The implementation for genotype calling tools into a screening or breeding program in agriculture requires extension of existing reliable, reproducible, automated calling methods in polyploid species. The development of such tools requires partnership among scientists and software developers. Toward this effort, iterative feedback and continued communication is critical among the scientific community and commercial partners. We will demonstrate currently available functionality in Illumina's GenomeStudio Genotyping Module on several polyploid plant species. We also suggest plans to extend automated support of polyploid calling. Goals for this presentation include facilitating dialogue with users to ensure that future development plans capture the needs of the community most thoroughly. The forum for this discussion in the International Triticieae Mapping Initiative is intended to fuel dialogue toward this software development effort due to the high demands of complex Triticeae genomes.

Oral Presentations

Session 5
Bioinformatics and Computational Biology

High-throughput data integration at URGI

D. Steinbach[1], M. Alaux[1], F. Alfama[1], J. Amselem[1], S. Arnoux[1], M. Bras[1], B. Brault[1], L. Brigitte[1], N. Choisne[1], S. Durand[1], A-F Fiston-Lavier[2], O. Inizan[1], V. Jamilloux[1], A. Keliet[1], E. Kimmel[1], N. Lapalu[1], I. Luyten[1], N. Mohellibi[1], C. Pommier[1], S. Reboux[1], D. Verdelet[1], D.Valdenaire[1], M. Zytnicki[1], H. Quesneville[2]

[1]URGI (Unité de Recherche Génomique-Info, INRA, Centre INRA de Versailles, RD 10, 78000, Versailles, France
[2]Department of Biology, Stanford University, Stanford, CA 94305- USA

Data integration is a key challenge of modern bioinformatics. It aims at providing biologists an intuitive way to explore data produced by different people in various projects. Indeed, large scale international projects generate lots of heterogeneous and unrelated data. The challenge becomes then to integrate and to link them with other publicly available data. Recent improvements in the throughput of nucleotide sequencing machines with the many applications in genomics and genetics fields critically emphasis on the necessity to have powerful information systems able to store, manipulate and explore these data. The URGI develops a such information system called GnpIS to bridge genetic and genomic data, allowing researchers to cross genetic information (i.e Genetic maps, QTL, markers, SNPs, germplasms, genotypes) with genomic data (i.e. physical maps, genome annotation, expression data) for species of agronomical interest. This system is in used for wheat data in the frame of many large INRA and international projects. The IWGSC has chosen this system to store their data (genomic sequence, annotation, physical maps). Tools developed at URGI allow efficient sequence annotation and data integration. As an illustration, we will present some results obtained on transposable elements (TEs), a key feature of wheat genome. In Arabidopsis, we showed that because of methylation spreading from TEs affecting neighbour genes, rates of TE evolution varies. Consequently, TEs located into gene-rich region evolve faster than in gene-poor regions, which make TEs appearing surprisingly younger and longer in gene-poor regions. Our results have important consequences for genome structure and evolution in TE-rich genomes such as the wheat.

Alphabet soup: some bioinformatic recipes for Triticeae

M. Spannagl, M. Martis, T. Nussbaumer, M. Pfeifer, H. Gundlach, K.F.X. Mayer [and dozens of colleagues from the International Triticeae community]

Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

The Triticeae genomes are challenging for assembling and finding/ordering genes due to their complex genome structures and high repeat content. We are tackling these problems with a variety of different techniques and approaches and aim to combine results to get a more in depth insight into Triticeae genome structures. Different routes are followed that provide independent yet complementary tracks towards the Triticeae genomes. One route is focused towards the development of an integrative approach that aims to link genetic and physical maps along with available sequence information with the goal to deliver a high resolution ordered gene map. A second route is based on a synteny driven integrative approach that is termed *GenomeZipper*. It has been successfully applied on a range of chromosomes and species and builds a foundation for a rapid yet highly robust access towards complex unsequenced genomes. Finally we exploit available large scale genomic and transcriptomic data in conjunction with model genomes to analyse for genes present, expanded and collapsed gene families, pseudogenes and genes that are specific for the Triticeae or individual species. We will present examples of the individual topics and outline how the individual building blocks complement each other in the aim to develop complete reference genomes.

QTL IciMapping v3.1: Integrated Software for Building Linkage Maps and Mapping Quantitative Trait Genes

J. Wang

Institute of Crop Science and CIMMYT China, Chinese Academy of Agricultural Sciences, No. 12, Zhongguancun South Street, Beijing 100081, China
E-mail: jkwang@cgiar.org or wangjk@caas.net.cn

QTL IciMapping is freely-available public software, capable of building high-density linkage genetic maps and mapping quantitative trait loci (QTL). There are six functionalities fully implemented in version 3.1, released in May 2011. 1. MAP: Construction of Genetic Linkage Maps in Biparental Populations. Grouping can be based on (i) anchored marker information, (ii) a threshold of LOD score, and (iii) a threshold of marker distance. Three ordering algorithms are (i) SER: SERiation, (ii) RECORD: REcombination Counting and ORDering, and (iii) MF: Multi-Fragment heuristic algorithm. Five rippling criteria are (i) SARF (Sum of Adjacent Recombination Frequencies), (ii) SAD (Sum of Adjacent Distances), (iii) SALOD (Sum of Adjacent LOD scores), (iv) COUNT (number of recombination events), and (v) LogL (Logarithm Likelihood of the marker sequence). 2. BIP: Mapping of Additive and Digenic Epistasis Genes in Biparental Populations. Five mapping methods are (i) SMA, Single Marker Analysis, (ii) SIM: the conventional Simple Interval Mapping, (iii) ICIM-ADD: Inclusive Composite Interval Mapping of ADDitive (and dominant) QTL, (iv) ICIM-EPI: Inclusive Composite Interval Mapping of digenic EPIstatic QTL, and (v) SGM: Selective Genotyping Mapping. 3. CSL: Mapping of Additive and Digenic Epistasis Genes with Chromosome Segment Substitution Lines Three mapping methods are (i) SMA: Single Marker Analysis, and (ii) RSTEP-LRT-ADD: Stepwise regression based likelihood ratio tests of additive QTL, and (iii) RSTEP-LRT-EPI: Stepwise regression based likelihood ratio tests of digenic epistasis QTL. 4. MET: QTL by Environment Interaction in Biparental Populations Two mapping methods are (i) ICIM-ADD: ICIM of additive QTL by environment interaction, and (ii) ICIM-EPI: ICIM of digenic epistatic QTL by environment interaction. 5. NAM: QTL Mapping in NAM Populations One mapping method is JICIM: Joint Inclusive Composite Interval Mapping of additive QTL. 6. SDL: Mapping of Segregation Distortion Loci in Biparental Populations Two mapping methods are (i) SMA: Segregation Distortion Loci Mapping through single marker analysis, and (ii) SIM: Segregation Distortion Loci Mapping through simple interval mapping.

Comprehensive analyses of 24,783 barley full-length cDNAs derived from 12 clone libraries

H. Sakai[1], T. Tanaka[1], H. Kanamori[2], K. Kurita[2], A. Kikuta[2], K. Kamiya[2], M. Yamamoto[2], H. Ikawa[2], S. Nakamura[3], N. Fujii[4], K.Hori[1, 5], T. Itoh[1], K. Sato[5], T.Matsumoto[1]

[1]National Institute of Agrobiological Sciences, Tsukuba, Japan
[2]Institute of Society for Techno-Innovation of Agriculture, Forestry, and Fisheries, Tsukuba, Japan
[3]National Institute of Crop Science, Tsukuba, Japan
[4]Hitachi Government and Public Corporation System Engineering, Ltd., Koto, Tokyo, Japan
[5]Okayama University, Okayama, Japan

Full-length cDNAs (FLcDNAs) are an essential resource for accurate gene structure annotation and biological characterization of organisms. In particular, FLcDNA clones expedites experimental studies of the biological functions of individual genes and their relationships. Here we report sequencing and analyses of a set of barley FLcDNA sequences that is composed of >172,000 clones derived from 12 libraries of a two-rowed malting barley, Haruna Nijo. After sequencing of both ends of the clones and their clustering, 24,783 clones were selected and further investigated as follows. First, those 24,783 FLcDNAs were completely sequenced, and were compared with ~5,000 publicly available barley FLcDNAs. As a result, 22,651 non-redundant FLcDNAs were obtained as barley representative sequences. Their characteristics, such as nucleotide lengths, completeness of predicted ORFs and functional classifications, were similar to those of rice FLcDNAs. We found that 3,278 were newly determined FLcDNAs because they showed no to known barley or wheat cDNAs. More than two thirds of these novel FLcDNAs were homologous to at least one of four monocots whose genome sequences were determined, so that their functions could be inferred from comparative analyses. We also found 1,699 barley-specific FLcDNAs that did not show any similarity against the four monocot genomes and wheat cDNAs. Second, a custom-made microarray with 30,653 probes from FLcDNA sequences were designed, and used for comprehensive transcriptome analysis. Experiments of 42 different conditions (two tissues, seven conditions and three time courses) revealed a correlation of gene expressions of three stress conditions and ABA treatments. In addition, more expression changes in root were observed than in shoot in five conditions. All of these sequences and their analyses are available at the Barley Full Length cDNA Database.

(http://barleyflc.dna.affrc.go.jp/hvdb/index.html).

GENESYS – A Gateway to Plant Genetic Resources

M. Mackay[1*], P. Brennan[2]

[1]Bioversity International, Via dei Tre Denari, 472/a, 00057 Maccarese, Rome, Italy
[2]CropGen International, Rock Valley, via Lismore 2480, Australia
[*]E-mail: M.Mackay@cgiar.org

Bioversity International (Bioversity), the Global Crop Diversity Trust (GCDT) and the Secretariat of the International Treaty on Plant Genetic Resources for Food and Agriculture (IT-PGRFA) have partnered to develop a global portal that facilitates access to and use of genebank accessions. The objective was to provide access to the passport data held in three significant online information systems SINGER (an initiative of the CGIAR System-wide Genetic Resources Programme - SGRP) , EURISCO (a web-based catalogue that provides information about *ex situ* plant collections maintained in Europe) and GRIN (the United States Department of Agriculture's Germplasm Resources Information Network), together with characterization, evaluation and environmental data from a range of providers, in a single portal. Called GENESYS (www.genesys-pgr.org), the portal represents a new paradigm in that it allows users to build custom queries to find accessions suitable for their research purposes using any combination of all available data. When launched in May 2011, GENESYS 1.0 contained some 2.33 million passport records, about 11 million characterization and evaluation records and over 11 million environmental records for accessions that have collecting site geo-references. This represents about one third of the estimated 7.3 million (SOW, FAO 2010) genebank accessions of plant genetic resources for food and agriculture. GENESYS also provides an intuitive interface that allows users to sift through accessions, using any available information, in the same way they think. For example, a wheat breeder might want to find white kernel accessions originating from a specific country where the annual precipitation is in a given range that are resistant or moderately resistant to a particular disease – this can be done, using GENESYS, with as few as six keystrokes and 12 mouse clicks. GENESYS also allows users to visualize collection site maps from a current query and download this data into Google® Earth for further analysis. Users can also create a query and then download the exact data fields they want for offline study. Finally, GENESYS also allows the user to make a request for available accessions directly to holding genebanks. GENESYS has demonstrated that a lot of data can be accessed through a single portal in the ways plant improvement scientists like to work. Inclusion of more data and functionality, including the ability to search information relating to non-genebank germplasm, are planned for the second phase of development. The immediate challenge is to engage genebanks, researchers and other data collectors and aggregators to share their information so additional value can be added to all germplasm made available through the portal.

Keywords: plant genetic resources, access, utilization, GENESYS

Oral Presentations

Session 6
New initiatives and technologies

SeeD: A Mexican initiative to characterize and broaden the genetic base of maize and wheat

P. Wenzl[1], E. Duveillier[1], M. Ellis[1], P. Figueroa[2], K. Mathews[1], M. Reynolds[1], C. Saint-Pierre[1], R. Shrestha[1], G. Soca[1], M. Bänziger[1]

[1]CIMMYT, Km 45 Carretera México-Veracruz, Texcoco, Edo. de México CP 56130, México
[2]INIFAP-CIRNO, Calle Dr. Norman E. Borlaug Km 12, Col. Valle del Yaqui Cajeme, Sonora CP 85000, México

The Seeds of Discovery (SeeD) initiative is one of four components of the Mexico-funded *Sustainable Modernization of Traditional Agriculture* program (MasAgro for its Spanish acronym). SeeD has the ambitious goal to comprehensively characterize the global genetic diversity of maize and wheat. The knowledge generated will provide a base for broadening the genetic base of these crops to develop high-yielding, climate-ready and resource-efficient cultivars. The principal objective of SeeD is to produce information-rich, easy-to-query catalogues for genebank collections in order to make them more accessible to breeders. To this end, SeeD will employ top-end IT tools to develop a user-oriented web portal that will help breeders to identify varieties with promising genetic composition, phenotypic traits, and environmental stress tolerances for their breeding programs. Genotyping-by-sequencing (GBS) will be used as the method of choice to estimate genetic relationships among genebank accessions without the ascertainment bias inherent in any fixed-array platform, while also generating sufficiently dense genome profiles for genome-wide association studies (GWAS). The allelic states of selected genes with known phenotypic effects will be evaluated or imputed from GBS profiles. SeeD outputs will also include a genetic-analysis service in Mexico which will not only generate most molecular data for the initiative, but will also assist breeders in the marker-assisted mobilization of novel, useful genetic variation into breeding programs. The SeeD strategy for wheat encompasses a systematic survey of the genetic relationships among the approximately 140,000 genebank accessions held at CIMMYT and selected accessions from other institutions; the preparation of new bread-wheat synthetics from previously under-sampled diversity pools; a grouping of genebank accessions according to phenological traits for subsequent phenotyping experiments; an exhaustive screen for heat and drought-tolerant accessions; an evaluation of pre-filtered subsets of accessions for other key characters such as P/N efficiency and resistance to wheat blast, rust, tan spot, *Septoria* and soil-borne diseases; GWAS for all evaluated traits; and the application of genomic-selection approaches to improve yield potential. Accessions with favorable genetic variation for investigated traits will be combined into multi-parent populations (stratified by phenology characters) to create a common resource for detailed genetic analyses. Selected genome regions with favorable effects on investigated traits will be introgressed into elite backgrounds, possibly by using genomic selection for genetically complex traits. All developed bio-assets developed by SeeD will be made openly available to users who are willing to commit to the open characterization, the unrestricted exchange, and the equitable use of the global maize and wheat genetic resources for the benefit of international agriculture.

The International Wheat Genome Sequencing Consortium (IWGSC): Building the Foundation for a Paradigm Shift in Wheat Breeding

K. Eversole

Executive Director, International Wheat Genome Sequencing Consortium, Eversole Associates, 5207 Wyoming Road, Bethesda, MD 20816, USA

As the staple food for 35% of the world's population and the most widely produced crop, wheat is one of the most important crop species. Genomics offers powerful tools for understanding the molecular basis of phenotypic variation, accelerating gene cloning and marker assisted selection, and for improving the efficiency of exploiting genetic diversity. In 2005, a group of wheat growers, breeders, and plant scientists launched the IWGSC (http://www.wheatgenome.org/) with the goal of securing a high quality, reference sequence of the bread wheat genome. The IWGSC's milestone-based strategy coupled with short and long-term roadmaps provide breeders access to an increasing array of tools and resources without having to wait for the completed physical maps or sequence. The development of a high quality whole genome physical map and whole genome reference sequence of the bread wheat genome remains a challenge as it is allohexaploid (2n=6x=42), highly repetitive (~80%), and 40 times the size of the rice genome with 17 billion base pairs. The IWGSC follows a chromosome-specific approach to develop in the first step, physical maps, low coverage sequencing, and high quality sequences of individual chromosomes before moving towards a gold standard reference sequence. Chromosome-based physical maps provide breeders immediate access to resources while also building the substrate for sequencing. In 2008, the completion of the physical map of the largest wheat chromosome (3B, ~ 1Gb) confirmed the feasibility of this approach. Development of the physical maps for the remaining chromosomes is underway. To facilitate anchoring, marker development, and to gain a first insight into the gene space composition, survey sequences were completed in conjunction with the construction of the physical maps. As survey sequences were available for half of the chromosomes by 2010, the IWGSC launched an internationally coordinated survey sequencing initiative that will provide breeders with survey sequences and the virtual gene order for all 21 chromosomes. High quality, minimum tiling path sequencing of the bread wheat genome began in 2010 at Genoscope (France) with chromosome 3B and funding for several other chromosomes is in place. An overview of the IWGSC strategies and activities will be presented.

Genotyping-by-sequencing for barley and wheat breeding and genetics

J. Poland[1, 2], P. Brown[3], B. Steuernagel[4], N. Stein[4], M. Sorrells[5], J.L. Jannink[5, 6]

[1]United States Department of Agriculture – Agricultural Research Service, Manhattan KS 66502 USA
[2]Department of Agronomy, Kansas State University, Manhattan KS 66502 USA
[3]Department of Crop Science, University of Illinois, Urbana IL 61801 USA
[4]Institute of Plant Genetics and Crop Plant Research Corrensstr. 3 D-06466 Gatersleben Germany
[5]Department of Plant Breeding and Genetics, Cornell University, Ithaca NY 14853 USA
[6]USDA-ARS, Ithaca NY 14853 USA

Rapid advances in sequencing technology have opened new possibilities for using genomic information in breeding and genetic studies in complex genomes such as barley and wheat. Genotyping-by-sequencing (GBS) uses reduced representation sequencing by targeting the genomic space flanking restriction enzyme sites. Combined with multiplexing samples using DNA barcoded adapters, this approach has enabled high-throughput genotyping with relatively low per sample costs. We have developed GBS protocols suitable for barley and wheat and have genotyped a range of mapping and breeding populations. We are using bi-parental populations to map GBS tags and SNPs and can currently discover and map between 5K and 30K SNPs per population. By combining different mapping populations we are developing a reference genetic map of sequence tags that will be useful for association genetics and genomic selection as well as anchoring and ordering genomic sequence. The GBS approach also facilitates the differentiation and mapping of duplicated sequences within large complex genomes. The utility of GBS for wheat and barley functional genomics will increase with advancements in the reference genome sequence and sequencing technology. We are currently using the GBS maps to anchor genomic sequence from whole-genome shotgun assemblies and BAC-ends. Anchoring of the GBS tags to the reference genome will provide an additional layer of genomic information, enabling identification of more polymorphisms. Improvements in sequencing technology will allow higher levels of multiplexing, driving per sample costs lower, further increasing the utility of this approach for genomics assisted breeding.

High throughput marker development from the repetitive fraction of the wheat genome

N. Cubizolles[1], C. Laugier[1], A.M. Mastrangelo[2], F. Choulet[1], P. Sourdille[1], F. Balfourier[1], L. Guerreiro[3], J. Gielen[4], J.P. Jaubertie[5], P. Jack[6], O. Robert[7], V. Korzun[8], P. Ouwerkerk[9], C. Feuillet[1], E. Paux[1].

[1]INRA UBP UMR1095 63100 Clermont-Ferrand, France
[2]Centro di Ricerca per la Cerealicoltura 71122 Foggia, Italy
[3]Arvalis-Institut du Végétal 75116 Paris, France
[4]Syngenta Seeds 31790 Saint Sauveur, France
[5]AgriObtentions 78041 Guyancourt, France
[6]RAGT Seeds Ickleton Essex CB10 1TA, UK
[7]Florimond Desprez 59930 La Chapelle d'Armentières, France
[8]KWS LOCHOW 37574 Einbeck, Germany
[9]Bayer BioScience NV B-9052 Zwijnaarde, Belgium

In wheat, the deployment of marker-assisted selection has long been hampered by the lack of markers compatible with high-throughput cost-effective genotyping techniques. In the past five years, many initiatives have been launched at the international level to develop SNP markers, mainly from genic regions. However, because of the reduced polymorphism level in coding regions and the low gene density, gene-derived SNPs do not allow to efficiently saturating the hexaploid wheat genome. By exploiting the repetitive fraction of the wheat genome, Insertion Site-Based Polymorphism (ISBP) markers can reach a density of one marker per 5-10 kb. In addition, ISBPs have been demonstrated to be an invaluable source of genome-specific SNPs that have the potential to complement gene-derived SNPs. The so-called ISBP-SNPs have been shown to meet the five main requirements for their utilization in marker-assisted selection: flexible and high-throughput detection methods, low quantity and quality of DNA required, low cost per assay, tight link to target loci and high level of polymorphism in breeding material. Based on these results, a public-private collaborative project called DIGITAL has been initiated, aiming at designing and genotyping ISBP-SNPs for marker-assisted breeding. In this context, we have implemented a new procedure for the high throughput discovery of ISBP-SNPs that led to the design of roughly 20,000 SNPs in elite cultivars. The genotyping of several thousands of these markers is underway and results will be used for both genetic mapping and association studies. In parallel, a new microarray-based genotyping technology called IMaGe (ISBP MicroArray Genotyping) has been developed that allows for the simultaneous scoring of several thousands of ISBPs. This technique has been used for genetic and physical mapping and diversity studies are being currently performed. Here we report on the most recent results of the DIGITAL project, including new technologies for SNP discovery and genotyping.

Next-generation tools for wheat genetics and breeding: high-throughput SNP genotyping assays and sequence-based genotyping

E. Akhunov[1], S. Chao[2], C. Saintenac[1], V. Catana[1], S. Kiani[1], D. See[3], G. Brown-Guedira[4], M. Sorrells[5], A. Akhunova[6], J. Dubcovsky[7]; C. Cavanagh[8], M. Hayden[9]

[1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA
[2]USDA-ARS Biosciences Research Laboratory, Fargo, ND, USA
[3]USDA Western Regional Small Grains Genotyping Lab, Johnson Hall, WSU, Pullman, WA, USA
[4]USDA-ARS Eastern Regional Small Grains Genotyping Lab., 4114 Williams Hall, NCSU, Raleigh, NC, USA
[5]Plant Breeding & Genetics, Cornell University, NY, USA
[6]Integrated Genomics Facility, Kansas State University, Manhattan, KS, USA
[7]Department of Plant Sciences, University of California, Davis, CA, USA
[8]CSIRO, Food Futures National Research Flagship, Canberra, ACT 2601, Australia
[9]Department of Primary Industries Victoria, Victorian AgriBiosciences Center, 1 Park Drive, Bundoora, VIC 3083, Australia

Genome-wide analysis of SNP variation is an extremely powerful approach for detecting genotype-phenotype associations and can be performed using high-throughput assays capable of detecting allelic variation in a predefined set of SNPs or by direct sequencing. The combined effort of several research groups in collaboration with the International Wheat SNP Working Group developed high-throughput SNP genotyping assays based on the Illumina iSelect platform. Next generation sequencing of transcriptomes was used for discovering gene-associated SNPs in the polyploid wheat genome. In a pilot experiment a set of 9,000 SNPs discovered in a population of 27 U.S. and Australian cultivars was used for designing the iSelect assay. Preliminary testing showed that more than 95% of SNPs produce high-quality genotype calls with up to 70% being polymorphic in a diverse sample of U.S. and Australian cultivars with a minor allele frequency >0.05. The assay is being used for studying the patterns of genetic diversity in a worldwide collection of wheat cultivars and for developing a high-density SNP map. An alternative approach to SNP detection relies on next-generation sequencing technologies for direct sequencing of complexity reduced genomic libraries prepared either by restriction digestion or by selective capture of genomic regions of interest. The latter strategy has been successfully used for studying single nucleotide and copy number variation in the exonic regions of the wheat genome. These newly developed tools now make possible to design experiments for detecting functionally relevant variation across the entire wheat genome and lay ground for the comprehensive analysis of genotype-phenotype relationships.

A 9K Infinium array for the characterization of barley lines and genetic mapping

M. Ganal[1], G. Durstewitz[1], J. Plieske[1], J. Russell[2], P. Hedley[2], M. Bayer[2], B. Kilian[3], N. Stein[3], R. Waugh[2]

[1]TraitGenetics GmbH, Am Schwabeplan 1b, 06466 Gatersleben, Germany
[2]James Hutton Institute, Invergowrie, Dundee DD2 5NE, United Kingdom
[3]Leibniz-Institute for Plant Genetics and Crop Plant Research, Correns str. 3, 06466 Gatersleben, Germany

An improved genotyping array for barley has been developed based on the Illumina Infinium technology. The array contains most of the previously developed SNPs from BOPA1 and 2 and a set of novel SNP markers identified from transcribed genes. Altogether, the array contains 9000 (9K) features which represent 7864 individual SNPs. Analysis of the array on several hundred barley varieties and lines permitted the development of a cluster file for reliable automatic allele calling in barley material for more than 6950 SNPs. The array has now been successfully used for genotyping large numbers of barley lines and mapping populations in a highly reliable way. Specific examples on the data quality analysis and use will be presented.

Presentation of an International Research Initiative for Wheat Improvement

H. Lucas[1], P.S. Baezinger[2], H-J. Braun[3], K. Eversole[4], C. Feuillet[5], P. Langridge[6], G. Moore[7]

[1]INRA, Département de Génétique et Amélioration des Plantes, Batiment 12, 78026, Versailles Cedex, France
[2]Department of Agronomy and Horticulture, 362D Plant Science Building, 1875 N. 38th Street, University of Nebraska, Lincoln, NE 68583-0915, USA
[3]CIMMYT, Km. 45, Carretera Mexico-Veracruz, El Batan, Texcoco, Edo. de México, CP 56130, México
[4]IWGSC-Eversole associâtes, 5207 Wyoming Road, 20816 Bethesda, MD,USA
[5]UMR INRA-UBP 1095 Génétique, Diversité et Ecophysiologie des Céréales Domaine de Crouelle 234, Avenue du Brézet 63100 Clermont-Ferrand, France
[6]ACPFG, PMB 1, Glen Osmond South Australia 5064
[7]Crop Genetics DepartmentJohn Innes Centre Norwich Research ParkColney Norwich NR4 7UH UK

An International Research Initiative for Wheat Improvement, bridging national research programmes and the international WHEAT programme coordinated by the CIMMYT was submitted early 2011 to the Agricultural G20, after endorsement by several national and international research and funding organisations. The main objectives of the International Research Initiative for Wheat Improvement, coordinated by an International Wheat Research Coordination Committee, are to:

- Coordinate world-wide bread and durum wheat improvement research efforts in the fields of genomics, genetics and agronomy, to increase food security, wheat nutritional value and safety in a rapidly changing environment while taking into account societal demands for sustainable and resilient agricultural production systems;
- Provide a forum to identify synergies and encourage collaborations among major nationally and internationally (public and private) funded wheat programmes with the result of maximising opportunities for gaining added-value internationally;
- Facilitate and ensure open communication and free, unencumbered exchange of germplasm, data, materials and ideas within the wheat research community;
- Support the development of publically available integrated databases and platforms;
- Recommend minimum data-reporting standards and develop protocols to allow consistency for screening and analyses;
- Organise knowledge transfer and capacity building;
- Monitor and summarize progress of scientific activities;
- Establish and periodically update priorities for wheat research of global relevance;
- Communicate to national and international funding agencies as well as to agricultural ministries the needs of the wheat research community of participating nations.

The initiative will be presented to the participants of the 2011 ITMI workshop. Implementation of the initiative will be discussed as well as the constitution of the International Wheat Research Coordination Committee.

Posters

Session 1
Structural and Functional Genomics

Chromosome genomics in wheat – where we are and where we go

J. Doležel[1], H. Šimková[1], J. Šafář[1], M. Kubaláková[1], I. Molnár[2], D. Edwards[3], K. Mayer[4], J. Rogers[5], K. Eversole[6], C. Feuillet[7]

[1]Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Sokolovská 6 CZ-77200 Olomouc Czech Republic
[2]Agricultural Research Institute of the Hungarian Academy of Sciences, H-2462, Martonvásár, Hungary
[3]University of Queensland, School of Agriculture and Food Sciences and Australian Centre for Plant Functional Genomics, Brisbane, QLD 4072 Australia
[4]Munich Information Center for Protein Sequences/Institute for Bioinformatics and Systems Biology, Helmholtz Center Munich, 85764 Neuherberg, Germany
[5]The Genome Analysis Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom
[6]Eversole Associates, 5207 Wyoming Road, Bethesda, MD 20816, USA
[7]Genetics, Diversity & Ecophysiology of Cereals, INRA-UBP 1095, 234, avenue du Brézet, 63100 Clermont-Ferrand, France

Chromosome genomics facilitates gene cloning and genome sequencing in bread wheat by dissecting the complex genome to single chromosome arms by flow cytometric sorting. BAC libraries constructed from DNA of isolated chromosomes have been used to develop sequence ready physical maps of wheat cv. Chinese Spring in the IWGSC-coordinated project in which various laboratories develop maps of individual chromosomes. Other applications of isolated chromosomes include physical mapping, targeted development of molecular markers and integration of genetic and physical maps. Rapid advances in next generation sequencing technologies provide tools to shot-gun sequence isolated chromosomes. Survey sequencing DNA amplified from isolated chromosomes enables characterization of chromosome gene space, DNA repeats and the targeted high throughput development of markers. Based on syntenic relationships between sequenced genomes and assembled chromosome specific sequence reads, genes can be placed on a chromosome in an approximate order and orientation. A collaborative project of the IWGSC aims to obtain survey sequences of all arms of cv. Chinese Spring during 2011. Current work to expand the utility of chromosome genomics in wheat focuses on three areas. The first involves chromosome sorting in wild relatives of wheat, including species of Aegilops. The results suggest the possibility of dissecting their genomes to single chromosomes and groups of chromosomes. This would open avenues for chromosome survey sequencing and comparative analyses in wheat, including the targeted development of markers to facilitate alien gene transfer. A second area focuses on developing a procedure for constructing optical maps of individual chromosome arms to support physical mapping and chromosome sequence assembly. Finally, the ability to purify single chromosomes from genotypes other than cv. Chinese Spring would facilitate SNP discovery and genotyping by sequencing in wheat. Preliminary results indicate that high-resolution flow cytometry makes it possible to obtain fractions significantly enriched for chromosomes of interest in any cultivar of wheat. Although the fractions may comprise a few other chromosomes, this approach offers a cost-effective way of reducing sequence complexity.

A piece of the bread wheat puzzle: the physical map of chromosome1BL

R. Philippe[1], E. Paux[1], P. Sourdille[1], I. Bertin[1], F. Choulet[1], C. Laugier[1], A. Korol[2], H. Bergès[3], S. Vautrin[3], S. Scalabrin[4], F. Cattonaro[4], J. Safar[5], H. Simkova[5], J. Dolezel[5], C. Feuillet[1]

[1]INRA, Genetic, Diversity and Ecophysiology of Cereals, Clermont-Ferrand, France
[2]University of Haifa, Institute of Evolution, Haifa, Israel
[3]INRA, Centre National de Ressources Génomiques Végétales, Toulouse, France
[4]IGA technology services, Udine, Italy
[5]Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic

The construction of the first physical map of chromosome 3B (Paux et al 2008) established a proof of concept for the feasibility of approaching the hexaploid bread wheat genome chromosome by chromosome. In the framework of the TriticeaeGenome project funded by the 7th European framework, a sequence ready physical map of chromosome 3B has been achieved (Rustenholz et al, 2011) and the physical mapping of three additional chromosomes: 1A, 1B and 3D is near completion. We have established the physical map of the long arm of chromosome 1B (1BL≈ 536Mb) using a 1BL specific BAC library of 15x coverage (92,640 clones). After fingerprinting with the SNaPshot technology and assembly using the FPC software and following the guideline established by the TriticeaeGenome project, 65,413 high quality fingerprints were automatically assembled into 1497 contigs with ≥ 5 BACs covering 94% of the 1BL chromosome. A Minimum Tiling Path (MTP) containing 8597 BACs was selected and three-dimensional MTP pools were established. To improve the physical map by manual merging in FPC and to order the physical contigs, we used 168 publicly available 1BL markers, 445 COS markers developed in the framework of the TriticeaeGenome project, and we developed 68,549 new markers (ISBP and SSR) from the 454 survey sequencing of the 1BL chromosome arm (1.2x). Among 1368 markers that were tested for their specificity, 515 1BL-specific markers were used to screen by PCR the 3D MTP pools and 10 deletion bin lines. In parallel, a 1BL neighbor genetic map containing 478 markers has been developed using 6 different genetic maps. To date, 412 contigs corresponding to 30% of the 1BL chromosome arm have been anchored in 9 deletion bins and 117 contigs representing 8% of the 1BL chromosome arm have been anchored on the 1BL neighbor genetic map. To improve further the physical map, we have developed a high-throughput approach based on the hybridization of the MTP on 2 different Nimblegen arrays: a 39,179 wheat unigenes array and a 17,788 ISBP array that was developed from the 1BL 454 survey sequences. An update of the results will be presented.

Contig anchoring of wheat 1BS using LTC analytical tools

Z. Frenkel[1], D. Raats[1], I. Dodek[1], T. Krugman[1], J. Dolezel[2], H. Simkova[2], H. Berges[3], F. Cattonaro[4], T. Wicker[5], E. Paux[3], C. Feuillet[3], T. Fahima[1], A. Korol[1]

[1]Institute of Evolution, University of Haifa, Haifa 31905, Israel
[2]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovska 6, Olomouc, CZ-77200, Czech Republic
[3]INRA, Genetics, Diversity and Ecophysiology of Cereals, Clermont-Ferrand, France
[4]Instituto di Genomica Applicata, Italy
[5]Institute of Plant Biology, University of Zürich, 8008 Zürich, Switzerland

Physical mapping of chromosome 1BS was conducted within the framework of the European consortium *TriticeaeGenome* designed to develop physical maps of wheat and barley group 1 and 3 chromosomes. Contig assembly of HICF fingerprinted BAC clones from 1BS specific BAC library was conducted using LTC program with liberal cutoff 10^{-25} of Sulston score. First assembly included 33,912 clones in 385 contigs (6-846 clones, each) covering 283 Mb (90% of 1BS). Then, contigs verified by parallel clone overlaps were assembled into 51 long supercontigs of ~1.2-22.3 Mb (average 5.3 Mb) using even more liberal cutoff 10^{-15} that cover about 85% of 1BS. Since the assembly of these supercontigs is less certain, we are currently conducting additional verification steps by using markers produced from BAC-end-sequences (BES) and by anchoring of the supercontigs to 1BS deletion bin map and 1BS Zipper, based on synteny with Brachypodium, Rice and Sorghum model genomes. In total, 62 *in silico* markers, based on BESs and 68 PCR markers developed based on genes and ESTs for wet lab tests, were used for anchoring of the supercontigs. Only 9 of 51 supercontigs have not yet been anchored to Brachypodium genome. Three long supercontigs of 10.5, 11.9 and 17.6 Mb in length were preliminary anchored to 1BS zipper by 8, 8 and 14 markers, respectively, well distributed along the supercontigs and in collinear order to their location in the Brachypodium genome. Further steps of anchoring and validation of supercontig are underway using high-throughput transcriptomic approach.

Chromosome 4D survey sequencing analysis: Present status and perspectives.

B. Clavijo[1], J. Wright[2], M. Martis[4], M. Caccamo[2], M. Valarik[3], F. Tabbita[5], J. Romero[6], L. Vanzetti[7], M. Bonafede[5], I. Garbus[6,8], M. Cativelli[5], P. Roncallo[6, 8], H. Simkova[3], K. Mayer[4], J. Dolezel[3], M. Tranquilli[5], N. Paniego[1, 8], V. Echenique[6, 8], M. Helguera[7]

[1]Instituto Nacional de Tecnología Agropecuaria/Instituto de Biotecnología 1686 Hurlingham Buenos Aires, Argentina
[2]The Genome Analysis Centre Norwich Research Park Colney Norwich NR4 7UH, UK
[3]Institute of Experimental Botany Sokolovska 6 CZ-77200 Olomouc, Czech Republic
[4]Munich Information Center for Protein Sequences/Institute for Bioinformatics and Systems Biology Helmholtz Zentrum Munich German Research Center for Environmental Health 85764 Neuherberg, Germany
[5]Instituto Nacional de Tecnología Agropecuaria/Instituto de Recursos Biológicos 1686 Hurlingham Buenos Aires, Argentina
[6]Departamento de Agronomía/ Universidad Nacional del Sur CERZOS/CONICET San Andrés 800, 8000 Bahía Blanca, Argentina
[7]Instituto Nacional de Tecnología Agropecuaria/EEA Marcos Juárez Ruta 12 S/N 2580 Marcos Juárez, Argentina
[8]Consejo Nacional de Investigaciones Científicas y Tecnicas, Argentina

Sequencing wheat genome has always been viewed as a complex task because of its large size, a high repetitive DNA content and polyploidy. Several of previously mentioned constrains (large size, polyploidy) can be overcome by flow sorting of individual chromosomes followed by shotgun sequencing using next generation sequencing (NGS) technologies. In this study we used 4D flow-sorted chromosome arms (obtained from cv Chinese Sping) to perform shotgun sequencing with a Roche 454 NGS platform producing sequence data equivalent to a 3.6x chromosome coverage. In order to reach a reliable and preliminary *de novo* assembly, short reads are being analyzed using different algorithms, strategies and parameters. Assembly performance is being evaluated by output comparison using rapid alignment tools to generate a correspondence relation between contigs on the assemblies. The identification of common (ie. emerging on various assemblies) and trustable (arguably emerging as better or more trustworthy variants when comparing different assemblies) contigs will allow downstream processes like molecular marker discovery and gene content evaluation using Genome Zipper. In the near future, additional multiplatform reads including pair end (considering Roche and Illumina technologies), will also be generated and evaluated to corroborate and enrich previous data.

Recent advances in physical mapping of the short arm of wheat chromosome 5A

V. Michelotti[1], K. Lacrima[1], F. Desiderio[1], D. Barabaschi[1], A. Gadaleta[2], I. Centomani[1], E. Francia[3], V. Mazzamurro[3], N. Pecchioni[3], A. Fricano[4], P. Piffanelli[4], S. Scalabrin$_5$, F. Cattonaro[5], A.M. Mastrangelo[6], J. Dolezel[7], A. Blanco[2], A.M. Stanca[1], L. Cattivelli[1], G. Valé[1]

[1]CRA–Genomics Research Centre, Via S. Protaso, 302, 29017 Fiorenzuola d'Arda (PC) – Italy
[2]Department of Environmental and Agro-Forestry Biology and Chemistry, University of Bari Via Amendola 165-A I-70126 Bari Italy
[3]Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia Via Amendola 2 I-42100 Reggio Emilia Italy
[4]Genomics Platform, Parco Tecnologico Padano Via Einstein I-26900 Località Cascina Codazza (LO) Italy
[5]Istituto di Genomica Applicata & IGA Technology Services SRL, Parco Scientifico e Tecnologico di Udine "Luigi Danieli", Via J. Linussio 51, I-33100 (UD), Italy
[6]CRA – Cereal Research Centre S.S. 16, km 675 I-71100 Foggia, Italy
[7]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovská 6, CZ-77200, Olomouc, Czech Republic

A high density genetic map is needed for anchoring BAC contigs during the construction of a physical map and for DNA sequence assembly. To undertake this challenge for wheat chromosome 5AS, we relied on four mapping populations: [1] Chinese Spring (CS, *Triticum aestivum*) x Renan (*T. aestivum*); [2] CS x CS disomic substitution line for chr. 5A (*T. turgidum* ssp *dicoccoides*); [3] Latino (*T. turgidum* ssp *durum*) x MG5323 (*T. turgidum* ssp *dicoccum*); [4] DV92 (*T. monococcum*) x G3116 (*T. monococcum*). Different parallel approaches for marker development were implemented: a) a DArT (diversity array technology) platform containing about 4000 new probes specific for 5A; b) a set of SSRs (simple sequence repeats) from databases and literature, and c) a set of COS (conserved ortholog set) and EST (expressed sequence tag) markers specific for 5A. One million reads of 400 base pairs in length (~2X) generated from 5AS flow sorted DNA, using the Roche 454 system, were screened in silico for SSR, and TE (trasponable elements) junction markers (ISBP, RJM and RJJM markers). After the assignment to chromosome 5A, performed using CS deletion and aneuploid lines, the markers have been tested for polymorphism between the four couples of parents, and accordingly, mapped in the corresponding populations. The integration of the resulting genetic linkage maps will produce a consensus map, essential tool to anchor the 4201 BAC clones of the MTP (minimal tiling path), produced thanks to the HICF (high information content fingerprinting) analysis of about 50.000 BACs derived from 5AS DNA. Additional specific markers are being developed from the sequencing of 2000 BAC-ends in order to improve the link between the genetic and physical map.

A strategy for the construction of physical map and pooled BAC-based sequencing of the wheat chromosome 6B

T. Matsumoto[1], F. Kobayashi[1], H. Kanamori[1], H. Mizuno[1], J. Wu[1], T. Itoh[1], Y. Katayose[1], J. Dolezel[5], S. Nasuda[2], T. Endo[2], K. Hayakawa[4], Y. Ogihara[3]

[1]National Institute of Agrobiological Sciences, 2-1-2, Kannondai, Tsukuba, Ibaraki 305-8602, Japan
[2]Lab of Plant Genet, Grad School of Agric, Kyoto Univ, Kyoto 606-01
[3]Kihara Institute for Biological Research, Yokohama City University, Maioka-cho 641-12, Yokohama 244-0813, Japan
[4]Nisshin Flour Milling Inc. 13 Ohkubo Tsukuba, Ibaraki 300-2611, Japan
[5]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Olomouc, Czech Republic

The International Wheat Genome Sequencing Consortium (IWGSC: http:// www.wheatgenome.org/) has been promoting to establish a high quality-physical map based reference sequence of the wheat genome with the coordination of national projects for genome sequencing. As a part of this activity, chromosome 6B(914Mb) has been assigned to Japanese research consortium that aims to accelerate sequencing of chromosome 6B of common wheat cultivar Chinese Spring. At present both the short and long arm part of chromosome 6B have been flow-sorted and chromosomal DNAs were collected using a double ditelosomic 6B line at IEB in Czech Republic. From these DNAs two BAC libraries have been established for physical map construction of short and long arm of chromosome 6B, respectively. Here we would like to explain our strategies for the simultaneous construction of BAC-based physical map and sequence assemblies. BAC contigs are constructed by sequence-based fingerprinting (WGP: Keygene/Amplicon Express Inc.). These contigs are screened for marker locations with the available molecular markers that are assigned to 6B, or homoeologous group 6 chromosomes (~1,000). We would also like to utilize the markers located in the syntenic position from other grass species (barley, rice, maize, Brachypodium, sorghum). Pooled BACs within a BAC contig will be sequenced by Roche/454 FLX Titanium pyrosequencers with 25x coverage, and resulting sequences will be assembled into sequence contigs. These sequences will again be searched for maker location, with which new BAC contigs could be recruited into the physical maps. The IWGSC promotes the chromosome survey sequences, in which we have shot-gun sequenced 6BS and 6BL with Illumina GAIIx sequencer (38GB, and 36GB, respectively). These sequences will help constructing a more comprehensive sequence scaffold that covers most of chromosome 6B of wheat. This work was supported partly by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics for Agricultural Innovation; KGS project) and by Nisshin Flour Milling Inc.

Construction of low coverage non-gridded BAC libraries for isolation of a genomic region involved in resistance to the Stem Rust in Wheat

S.Vautrin[1], I. Mercier[1], A. Bellec[1], G. Beydon[1], E. Prat[1], R.Mago[2], W. Spielmeyer[2], H. Bergès[1*]

[1]INRA/CNRGV - Chemin de Borde Rouge - 31326 - Castanet-Tolosan - France
[2]CSIRO - GPO Box 1600 - Canberra, ACT 2601 - Australia
[*]E-mail: hberges@toulouse.inra.fr

The French Plant Genomic Resource Centre (CNRGV), dedicated to plant genomics, is in charge of more than 8 millions unique samples among more than 50 plant genomic libraries of model and crop plant. The objectives of the CNRGV are to provide high throughput molecular tools to the scientific community. BAC libraries are an invaluable tool for genome analysis, physical mapping, map-based cloning and sequencing projects. They facilitated gene cloning and contributed to rapidly identify homoeologous genes in polyploïd species. In order to focus directly on a genomic region of interest in specific genotypes and rapidly isolate BAC clones spanning a genomic region, we have developed a non gridded BAC library approach. This method permits to avoid time and cost expensive steps of numerous BAC clones re-arraying and screening, and an efficient access to sequence diversity among plant cultivars in specific genomic region. For several different plants species, we are now constructing BAC library from genotypes of interest, starting with a 1x genome coverage. Directly after transformation, all the clones are shared in several pools which are screened using specific PCR markers. The BAC clones carrying the markers are individualized by picking and screening a minimal number of clones issued from a positive pool. In case of no positive pool identified, one or more additive steps of transformation, pooling and screening can be processed, until BAC clones located on the region of interest are found. After further validation steps, pools of tagged BACs can be finally sequenced using Next Generation DNA Sequencing technology (454 technology). This strategy has proven to be an efficient way to directly target genomic region of interest and explore the variability among specific genotypes. An update on a project aiming at the characterization of the sr2 locus in Bread Wheat “Hope” line involved in durable resistance to the wheat stem rust pathogen, will be presented.

Evaluation of the synteny for 5AS chromosome in *triticum* species with different ploidy levels

K. Lacrima[1], V. Michelotti[1], F. Desiderio[1], D. Barabaschi[1], A. Gadaleta[2], I. Centomani[1], A. Giancaspro[2], S. Giove, E. Francia[3], V. Mazzamurro[3], N. Pecchioni[3], A. Fricano[4], P. Piffanelli[4], A.M. Mastrangelo[5], M. Valarik[6], A. Blanco[2], A.M. Stanca[1], L. Cattivelli[1], G. Valé[1]

[1]CRA–Genomics Research Centre, Via S. Protaso, 302, 29017 Fiorenzuola d'Arda (PC) – Italy
[2]Department of Environmental and Agro-Forestry Biology and Chemistry, University of Bari Via Amendola 165-A I-70126 Bari Italy
[3]Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia Via Amendola 2 I-42100 Reggio Emilia Italy
[4]Genomics Platform, Parco Tecnologico Padano Via Einstein I-26900 Località Cascina Codazza (LO) Italy
[5]CRA – Cereal Research Centre S.S. 16, km 675 I-71100 Foggia Italy
[6]Institute of Experimental Botany, laboratory of Molecular Cytogenetics and Cytometry, Sokolovska 6, 772 00 Olomouc Czech Republic

In the frame of the project "Physical mapping of wheat chromosome 5A", we have undertaken an investigation about the synteny level of the short arm of chromosome 5A (5AS) among different species of *Triticum* genus characterized by a different ploidy level and evolutionarily separated on a time scale in order to get insights into possible chromosomal rearrangements occurred during evolution. The analyzed species were *Triticum aestivum* (AABBDD; 2n=42), *Triticum durum* (AABB; 2n=28) and *Triticum monococcum* (AA; 2n=14). In details, we relied on four mapping populations: [1] Chinese Spring (CS, *T.aestivum*) x Renan (*T.aestivum*); [2] CS x CS disomic substitution line for chr. 5A (*T. turgidum ssp dicoccoides)*; [3] Latino (*T. turgidum ssp durum*) x MG5323 (*T. turgidum ssp dicoccum*); [4] DV92 (*T. monococcum*) x G3116 (*T. monococcum*). High density genetic maps have been developed for the short arm of wheat chromosome 5A in these four populations using SSR (simple sequence repeat), SSR-EST (SSR-expressed sequence tags), TE junction (trasponable elements) and COS (conserved ortholog set) comparative anchor markers. The specificity of these markers for chromosome 5AS has been assayed using nulli-tetrasomic lines derived from the reference cultivar Chinese Spring. Moreover the physical position of the developed markers has been assigned to deletion bins of 5AS through the utilization of deletion lines. The evaluation of syntenic blocks and non-conserved regions among the homologous segments of different *Triticum* species is reported, while the mapping of EST-based markers allowed identification of syntenic regions in the rice and brachypodium genomes. Identification of possible rearrangements in the different 5AS genetic maps of wheat provides valuable information about the subsequent steps on the BAC contigs anchoring while the consensus map deriving from the integration of these four maps will provide a fundamental tool to link the genetic and physical maps.

The cell wall invertase (IVR1) gene from wheat

H. Webster, J. Fosu-Nyarko, G. Keeble, P. Moolhuijzen, M. Bellgard, B. Dell, R. Appels

State Agricultural Biotechnology Centre, Murdoch University, Perth Western Australia, 6150

Genome level sequencing has established the detailed structure for the IVR1 genes on 1A (Francki et al 2006) and two genes on the genome segment ctg506 from chromosome 3B. The IVR1 gene is involved in sugar transport to developing pollen and is a key point in the sugar transduction pathway that can be down regulated by water stress, thus resulting in aborted pollen development and ultimately reduced grain set (Ji et al 2010). In wheat, susceptibility to drought stress during reproductive stage growth is under polygenic control and also shows marked varietal variation. Thus detailed studies of the structure of the gene and identification of its location within the reproductive stage water stress QTL is an important step towards improving the efficiency of marker assisted selection for germplasm with the genetic potential to confer tolerance to this stress. Analysis of the structure of the IVR1 genes has shown the sequence is highly conserved between the A, B, and D genomes and between the varieties Westonia and Kauz that were crossed to generate a population of 230 double haploid lines (W x K DH lines). A high degree of sequence homology between IVR1 and a closely related cell wall invertase IVR1.2 was also evident. Comparisons between the IVR1 genes and the equivalent genes in rice and Brachypodium are providing insights into the relationship between gene structure and function. Phenotype characterisation of the WxK DH's has revealed distinct groupings with regards to plant height and maturity time and both of these characteristics may be linked to the expression of the different IVR1 genes under water stress conditions. Water stress studies are being carried out to evaluate the relative effect of phenotype on the expression of IVR1 in order to provide basic data enabling better understanding of the genetic mechanism underpinning varietal variability in expression of the gene.

Functional Characterization of a Heat Stress Induced Heat Shock Transcription Factor from Wheat

P. Khurana, H. Chauhan

Department of Plant Molecular Biology, University of Delhi South Campus, Dhaula Kuan, New Delhi-110021, India

Because wheat is a temperate annual, it is not optimally suited to growth in many parts of the world's wheat growing areas, where temperature during grain filling may reach 40^{o}C. High temperature stress affects almost every stage of wheat development from germination to grain filling. Through Suppression Subtractive Hybridization a total of 5500 clones were identified to be differentially expressed. Apart from many novel genes, a large number of transcription factors that were found to be up-regulated by high temperature. A novel seed specific heat shock factor (HSF) inducible by heat stress was identified in developing wheat seed tissues. This seed specific wheat HSF was cloned and characterized by directional genome walking technology following using 5' and 3' RACE. The expression of this heat shock factor was also checked by real time PCR analysis. *TaHSF* was found to be induced by calcium and heat stress, particularly in various flower and seed tissues, and significant amounts were present in 4-day recovery tissues, suggesting its dual role in stress amelioration and as well as in seed maturation. It was also found to be induced by salt stress, ABA and PEG. Transgenic *Arabidopsis* plants of *TaHSF* gene were also raised. As was evident from the growth and biomass accumulation, overexpression lines showed much better response as compared to wild type. A mutant line lacking HSF showed reduced growth and less biomass, whereas the complemented line showed biomass accumulation and growth pattern almost similar to that of wild type.

Spatiotemporal expression profiling of WRKY78 gene during wheat-leaf rust interaction

D. Kumar, D. Singh, M. Kumar, R. Bandopadhyay, K. Mukhopadhyay

Department of Biotechnology, Birla Institute of Technology, Mesra, Ranchi 835215, Jharkhand, India

The plant WRKY proteins are among the ten largest families of transcription factors having the recognition sequence (T)(T)TGAC(C/T), known as the W-box, which is found in the promoter regions of WRKY and other defense-related genes. They play regulatory roles in transcriptional reprogramming associated with several physiological processes like biotic and abiotic stress as well as biosynthesis of secondary metabolites. WRKY gene family are subdivided into three different groups, group I, II and III, based on the number of WRKY domains. Wheat (*Triticum aestivum* L) contains more than one WRKY genes due to its hexaploid nature but very little is known on these transcription factors in wheat. To understand the role of WRKY transcription factors during wheat-leaf rust interaction, we have designed primers from the consensus sequences obtained from multiple sequence alignment of wheat WRKY sequences available at NCBI database. Genomic and cDNA sequences were amplified from resistant (HD2329+Lr28) and susceptible (HD2329) near isogenic wheat lines and the sequences were analyzed using various bioinformatics softwares. Multiple sequence alignment was performed with other wheat WRKY sequences from NCBI database and a phylogenetic tree was constructed. Temporal and spatial expression profiling of the WRKY gene were done by quantitative real-time PCR using WRKY specific primers and probe from RNA isolated from resistant and susceptible plants during mock and infected condition. Transcript level normalization was carried out using wheat *Actin*. The study revealed maximum expression of WRKY78 gene at 24 and 48 hours post inoculation (hpi) in both compatible and incompatible interaction.

Molecular and physiological characterization of a light-green durum wheat mutant

A.Peremarti[1], D. Villegas[1], P. Christou[2], T. Capell[2], C. Royo[1]

[1]Institut de Recerca i Tecnologia Agroalimentàries (IRTA). Av. Alcalde Rovira Roure 191. 25198. Lleida, Spain
[2]Dept. Producció Vegetal i Ciència Forestal. Universitat de Lleida. Av. Alcalde Rovira Roure 191. 25198. Lleida, Spain

Chlorophyll content in cereal crops determines the amount of solar radiation absorbed by the canopy, thus controlling the photosynthetic capacity and the transpiration efficiency, with important effects on the potential yield. The light-green mutant MD597, derived from the durum wheat (*Triticum turgidum* subsp. *durum*) *cv.* Borgia (WT) after a mutagenic treatment with sodium azide (N_3Na), was characterized at physiological and molecular levels, and tested in field and glasshouse experiments. Transcriptomic and proteomic approaches were used in an attempt to identify differently regulated genes. Compared with the WT, the microscopic structure of the mutant leaves showed a disorganized pattern on the grana distribution. Chlorophyll (Chl) and carotenoid content at anthesis were 42.5% and 41% lower in the mutant than in the WT, respectively. The leaves of the mutant showed the largest reduction in Chl content than the sheath, stem or spikes, being Chlb more reduced than Chla in comparison with the WT. Lutein was, among the carotenoids, the most drastically reduced in the mutant. Three differentially expressed proteins: 2-Cys peroxiredoxin (BAS1) and two chlorophyll a-b binding proteins (CAB) were identified in the mutant by the proteomic analysis of leaf tissues at tillering. 2-Cys peroxiredoxins are chloroplastic peroxidases that can modulate redox signaling during development and adaptation, and can regulate metabolism in thylakoids. CAB proteins form part of the light-harvesting antenna (Lhc). Transcriptomic analysis was unable to identify any differentially expressed gene between Borgia and the mutant in plant samples taken at tillering. Discrepancies in SPAD units between Borgia and MD597 were larger at seedling and tillering than at heading and anthesis both in field and glasshouse experiments. Differences among both genotypes in the physiological behavior after anthesis were maximized under severe drought stress conditions. Preliminary results suggest lower levels of photo-inhibition and light saturation, and better capacity of heat dissipation in the mutant than in the WT.

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Molecular characterization of two novel Glu-1B encoded subunits from Kotte (*Triticum aestivum* L.)

E. Gregová[1], L. Leišová-Svobodová[2], V. Šudyová[1], S. Šliková[1]

[1]Plant Production Research Center Piešťany, Bratislavská cesta 122, Slovak Republic
[2]Crop Research Institute, Drnovská 507, Prague 161 06, Czech Republic

Glutenins were composed of high-molecular-weight (HMW) subunits and low-molecular-weight (LMW) subunits. High-molecular-weight glutenin subunits (HMW-GSs) are the most important seed storage proteins determines bread making quality of wheat flour. In hexaploid wheat (*Triticum aestivum* L.) the genes controlling the synthesis of glutenins are located on the chromosomes 1A, 1B and 1D but genes controlling the synthesis of HMW glutenins are located on the long arms of the chromosomes in the loci *Glu-1A*, *Glu-1B* and *Glu-1D*. Each of the loci *Glu-1A*, *Glu-1B* and *Glu-1D* contains tightly linked the genes x and y, which are related but have subtle differences in their structures and properties. Two novel subunits (designated 1Bx6.1 and 1By8.1, respectively) at the *Glu-B1* locus were identified in the Sweden wheat landrace 'Kotte' by comparison of subunit mobility with that previously identified in several standard hexaploid wheats. The 1Bx6.1 and 1By8.1 genes were isolated using locus-specific PCR primers and the sequence of complete open reading frames (ORFs) were obtained. Subunit 1Bx6.1 consists of 2478 bp encoding a premature protein of 661 amino acid residues, whereas By8.1 consists of 2155 bp encoding a premature protein of 586 amino acid residues.

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Posters

Session 2
Mapping and Cloning

Re-localization of the gene for crossability on chromosome 5B of common wheat

K. Mishina[1, 2], Y. Shirai[1], M. Katsumata[1], H. Sassa[1], T. Koba[1]

[1]Graduate School of Horticulture, Chiba University 648 Matsudo Chiba 271-8510 Japan,
[2]Laboratory of Plant Genome Science, Kihara Institute for Biological Research, Yokohama City University Maioka 641-12 Totsuka Yokohama 244-0813 Japan

Crossability of common wheat is one of the reproduction barriers in wide crosses in Triticeae. The crossability gene, *Kr1* was previously mapped on the long arm of chromosome 5B by telosomic mapping method. We tried genetic mapping using three RIL populations derived from crosses between Chinese Spring (high crossability) and chromosome substitution lines of Chinese Spring having chromosome 5Bs of Cheyenne, Mara and Hope (low crossability) to detect varietal difference. The degrees of the crossability were measured by the ratios of hybrid seed set with rye. In these populations, major QTL regions controlling crossability with rye were detected on the distal region of the short arm of each chromosome 5B, supposed to be *Skr* locus. SSR markers, *cfb306*, *cfb341*, and *gwm234* were tightly linked to the crossability as a single locus. Especially in the population of Hope 5B, which was previously used for telosomic mapping, the locus for the crossability was mapped on chromosome arm 5BS. Each RIL having distal fragment of 5BS from each low crossability cultivar showed significant decrease of rye pollen tube elongation. Therefore, *Kr1* gene, a major factor for the crossability on 5B, is thought to be corresponding to *Skr* locus.

Map-based cloning of SKr, a major QTL involved in crossability of wheat with rye

T. Gerjets[1], S. Hofmann[1], P. Sourdille[1], G. Gay[1], F. Choulet[1], S. Vautrin[2], H. Bergès[2], A. Bouguennec[1], F. Balfourier[1], W. Alfares[1,3], P. Barret[1], C. Feuillet[1]

[1]UMR INRA-UBP 1095 Genetics, Diversity and Ecophysiology of Cereals (GDEC) 234 Avenue du Brezet, 63100 Clermont-Ferrand, France
[2]INRA CNRGV Chemin de Borde Rouge BP 52627, 31326 Castanet Tolosan, France
[3]Aleppo University/Faculty of Agriculture, Aleppo, Syria

Most modern bread wheat varieties (*Triticum aestivum* L.) are non-crossable with related species such as rye (*Secale secale* L.), thereby reducing the possibilities to reintroduce genetic diversity in the elite wheat gene pool through intra- and interspecific crosses. Only ancient wheat and landraces are crossable, in particular those originating from the Asian gene pool.
In 1998, a strong QTL, called SKr, underlying crossability of wheat with rye, was mapped at the distal end of chromosome 5BS (Tixier *et al*, 1998, Lamoureux *et al* 2002). High association was observed between the crossability and the alleles from the crossable lines at the SKr locus, whereas no correlation was detected with the Kr1 locus which is located on the long arm of chromosome 5B. The major effect of SKr on crossability in wheat could provide very useful markers for efficient introgression of SKr alleles in elite wheat varieties to increase the genetic diversity in wheat and triticale breeding programs. Using a detailed genetic map established in a HIF population of 223 individuals originating from a cross between the non-crossable French variety Courtot and the crossable variety Chinese Spring, SKr was mapped in a 4.6 cM interval. Physical maps of the SKr locus are currently under construction in both Chinese Spring and Courtot using whole genome BAC libraries. To date, 6 markers have been developed in the genetic interval using shotgun sequences from wheat BAC clones identified by chromosome walking as well as orthologous relationships with rice. BAC contigs have been established at the SKr locus in Chinese Spring and Courtot. Gaining access to both crossable and non-crossable backgrounds will guarantee the isolation of the SKr gene controlling the inhibition of crossability, even if the recessive trait of crossability in Chinese Spring originates from a deletion. Recently, more than 200 lines from various gene pools (European, Asian) have been phenotyped and genotyped to prepare the foundation for performing association studies with candidate genes and for analyzing the spatio-temporal origin of crossability. An update of the project will be presented.

Compositum barley – towards genetic elucidation of gene(s) regulating inflorescence branching

T. Seidensticker[1], L. Rossini[2], T. Schnurbusch[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genebank Department, Plant Architectur, Corrensstr. 3, 06466 Gatersleben, Germany
[2]Dipartimento di Produzione Vegetale, Via Celoria 2, 20133 Milano, Italy

Consolidated knowledge related to the molecular-genetic mechanisms underlying inflorescence development and growth in cereal grasses is crucial to understand and improve their reproductive success. While developmental mutants have been extensively investigated in several plant species, only a few genes have been isolated in barley following this approach. The non-branched inflorescence axis is one of the most noticeable characteristics of barley compared with other grasses like rice and maize. The inflorescence mutants, known as *compositum* type, of our barley collection show spikes with elongated rachillas that occur mainly in the basal part of the spike and form rachis-like branches, and thus generating a ramified spike. Anatomical differences of young barley apices between wild type and *compositum2*-mutants *(com2)* were analyzed using scanning electron microscopy to identify developmental variation at different immature spike stages. Our analyses exhibited abnormal spike morphology of branched mutants even during early stages of development. Here, an apparent loss of spikelet determinacy after the initiation of glume primordia may have resulted in an elongation of the spikelet meristem and the formation of ectopic branches. From past barley mutant analysis it was known that the branching mutant (*brc1*) has been mapped to the short arm of chromosome 2H, but so far no further refined mapping has been described and no branching mutants from barley have been molecularly identified. In order to elucidate inflorescence branching in barley we utilized the available information on whole-genome sequences of grasses like rice, maize and *Brachypodium* for mapping, molecular marker development and candidate gene approaches. The latest results on the phenotypic analysis of the early development of branching and more detailed mapping of the branching locus *com2* in barley will be presented.

Towards the molecular elucidation of 'Miracle Wheat' – wheats with branched inflorescences

T. Seidensticker[1], A. Börner[2], T. Schnurbusch[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany, Genebank Department, [1]Plant Architecture
[2]Resources Genetics and Reproduction

Inflorescences of grass species have a distinct morphology in which cereal grains are produced in specific plant organs called spikes that are constituted of spikelets attached to the rachis. The development of spikes in cereal crop plants is one of the important determinants of their reproductive success. Hence, understanding the molecular and genetic mechanisms that regulate key morphological and developmental traits such as inflorescence architecture, spikelet initiation and abortion is crucial but almost completely lacking in most of our cereal crops. Our wheat collection with distinct spike phenotypes includes mutants with branched inflorescences (so called 'Miracle Wheats') which show indeterminate spikelet growth i.e. loss of terminal spikelet formation. These wheat mutants develop branches of second order spikes also indeterminate in growth but primarily originating from the more basal parts of the primary spike. Moreover, such spikes often produce more than one spikelet per rachis node, resulting in double or even triple spikelet development (supernumerary spikelets). Such deviant inflorescence features, however, are usually associated with an increased grain number per spike and might deliver a basis for breeding and potentially enhancing future yields of wheat crops and related grasses. In previous studies the branched-spike phenotype in tetraploid wheats has been linked to the short arm of chromosome 2A; however a refined map location for the *branched head* (*bh*) locus has not yet been presented. In order to elucidate inflorescence branching within the tribe Triticeae we used around 650 F2 individuals and around 25 polymorphic microsatellite markers from three mapping populations between 'Miracle Wheat' and standard durum wheat (*T. turgidum* L. subsp. *durum* (Desf.) Husn.) cultivars and confirmed its chromosomal location on 2AS.

Application of Infinium 9K SNP genotyping for bulk segregant analysis in wheat

M.J. Hayden[1], K.L. Forrest[1], S.L. Kong[1], D. Wong[1], S. Chao[2], C. Cavangah[3], E. Akhunov[4]

[1]Department of Primary Industries Victoria, Victorian AgriBiosciences Center, 1 Park Drive, Bundoora, VIC 3083, Australia
[2]USDA-ARS Biosciences Research Laboratory, Fargo, ND, USA
[3]CSIRO, Food Futures National Research Flagship, Canberra, ACT 2601, Australia
[4]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA

Since its inception in 1991, bulk segregant analysis has had widespread application in wheat research. It has been used both for rapid identification of trait linked markers and to saturate genomic regions of interest with molecular markers. The wheat Infinium 9K SNP genotyping assay, developed by the International Wheat SNP Working Group, now makes it possible to combine bulk segregant analysis with high-density, genome-wide SNP genotyping, thereby opening possibilities for affordable routine and highly sensitive trait-linked marker detection. Here, we describe the results of an investigation to determine optimal parameters for deploying Infinium 9K SNP genotyping in bulk segregant analysis, and the development of a highly automated pipeline for identifying markers linked to genomic region of interests.

Further characterization of the phenotypic effects associated to *QYld.idw-3B*, a major QTL for grain yield in durum wheat, using a set of near isogenic lines

M. Graziani[1], M. Maccaferri[1], A. Massi[2], E. Paux[3], C. Feuillet[3], S. Castelletti[1], M.C. Sanguineti[1], R. Tuberosa[1].

[1]Department of Agroenvironmental Science and Technology, Viale Fanin 44, University of Bologna, 40127 Bologna, Italy
[2]Società Produttori Sementi Bologna (PSB), Via Macero 1,40050 Argelato (BO), Italy
[3]Institut National de la Recherche Agronomique, UMR INRA-UBP 1095 Génétique, Diversité et Ecophysiologie des Céréales, Domaine de Crouelle 234, 63100 Clermont-Ferrand, France

A major QTL for grain yield *per se* (*QYld.idw-3B*) was identified in a durum wheat recombinant inbred line population derived from Kofa × Svevo (Maccaferri *et al*. 2008, Genetics 178: 489-511). Presently, the fine mapping of *QYld.idw-3B* as well as its further phenotypic characterization is underway in the framework of the FP7 TriticeaeGenome Project. A total of 44 new markers (BAC-derived SSR, ISBP and SNP markers anchored to the Chinese Spring physical map) have been added to the target interval (12 cM). Nineteen pairs of near-isogenic lines (NILs) were obtained from $F_{4:5}$ heterogeneous inbred families. Based on their graphical genotypes, the NILs pairs were categorized into four groups characterized by homogeneous contrasting haplotypes of different length at the QTL region. All NIL pairs were evaluated in field trials carried in 2010 at two sowing densities (200 and 400 viable seeds per square meter. Morpho-physiological traits, grain yield and yield components were recorded. ANOVA revealed highly significant differences between the lines with the Kofa allele (+/+) and those with the Svevo allele (-/-) for most of the investigated traits while non significant interaction effects were observed with sowing density. The previously detected effects of *QYld.idw-3B* on grain yield, plant height, peduncle length and kernel weight were confirmed in most NIL pairs. Within each pair of NILs, the +/+ and the -/- line did not show any appreciable morpho-physiological difference in terms of plant vigour, tillering and leaf greenness up to the booting stage, while, starting from this stage, some important differences were detected. In fact, as compared to the the -/- lines, the +/+ lines headed earlier, showed a longer ear peduncle and plant height, a higher total dry weight at anthesis and a higher grain yield. The combined analysis of the phenotypic and molecular data allowed us to to assign the QTL peak to Contig 954, a 3.1 Mb region. Fine mapping will be carried out by genotyping a new set of $F_{4:5}$ recombinant segmental isolines with all the available markers.

Genetic dissection and molecular breeding for drought resistance in some Indian wheat cultivars

V. Gahlaut, S. Tyagi[1], B.S. Tyagi, G. Singh, S. Dreisigacker, P. Langridge, H.S. Balyan, P.K. Gupta

[1]Molecular Biology Laboratory, Department of Genetics and Plant Breeding, Ch. Charan Singh University, Meerut - 250 004, UP, India

In order to deal with the problem of decline in wheat production due to drought, we initiated a comprehensive plan to study the genetics of drought tolerance and to improve drought tolerance through molecular breeding in some Indian wheat cultivars. QTL interval mapping involving two mapping populations developed in Australia, and association mapping involving 330 diverse accessions of a spring wheat nursery (developed under Generation Challenge Programme, and supplied by Susanne Dreisigacker) are being used for dissecting the genetics of drought tolerance. The genes involved in 'two-component system', namely histidine kinase (HK), histidine-containing phosphotransfer (HPt) and response regulator (RR) will also be used to study the genetics of drought tolerance in wheat. Phenotyping data has already been recorded on one of the two Australian DH mapping populations (received from Australia by DWR, Karnal), namely Excalibur × Kukri that was grown at three drought-prone locations in India (the data for the other mapping population, i.e. RAC875 × Kukri will be collected next year). Using this phenotypic data and the marker genotyping data kindly supplied by Peter Langridge, QTL interval mapping will be conducted and results presented. Similarly, the spring wheat nursery of 330 diverse genotypes was grown under rain-fed conditions at Meerut (India) and relevant phenotyping data was recorded. Using this phenotyping data along with the genotyping data to be received from Susanne Dreisigacker of GCP, association mapping will be completed and presented. Hopefully, these two approaches should give valuable information about the genetic architecture involved in drought tolerance in wheat. In addition to the above, five crosses, each involving a drought tolerant and a drought susceptible high yielding genotype, were attempted in 2010 summer using off-season nursery grown at Keylong (India). Backcrosses were made in the rabi 2010-11. BC_1F_1 population will be raised in rabi crop season 2011-12, and MAS will be exercised using known marker-trait associations (including markers developed using interval mapping and association mapping in the present study) in combination with phenotypic selection. The known markers will also be validated using bulk segregant analysis (BSA). This should eventually lead to the development of some drought tolerant genotypes suitable for cultivation in India.

Identification of barley *Mat-a* as *Early Flowering 3* (make it more scientific less commercial)

S. Zakhrabekova[1], S.P. Gough[1], I. Braumann[1], A. Müller[1], J. Lundqvist[1], K. Ahmann[1], C. Dockter[1], I. Matyszczak[1], M. Kurowska[2], A. Druka[3], R. Waugh[3], A. Graner[4], N. Stein[4], U. Lundqvist[5], M. Hansson[1]

[1]Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-1799 Copenhagen V, Denmark,
[2]Department of Genetics, Faculty of Biology and Environment Protection, University of Silesia, Jagiellonska 28, 40-032 Katowice, Poland
[3]Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom
[4]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466 Gatersleben, Germany
[5]The Nordic Genetic Resource Center, Smedjevägen 3, SE–230 53, Alnarp, Sweden

The molecular mechanism behind timing of flowering is rapidly emerging, illuminated mainly from studies with *Arabidopsis thaliana* mutants. Timing of flowering is regulated by seasonal cues like temperature and photoperiod and tightly connected to vernalization and the circadian clock. Already in the 1930s plant breeders realized the importance of early flowering. This target may limit high productivity in fertile areas because early cultivars do not use the entire growing season. However, early traits allow less fertile areas and environments with short growing season to be used for agricultural output. Over the years more than 1200 early barley mutants were isolated at the Swedish Seed Association. The mutants could be grouped into three different categories differing in heading time with a variation between one and ten days. Allelism tests were conducted with 177 of the mutants and 9 different loci could be established. The *mat-a* group was the largest, consisting of 84 alleles. It was further established that *mat-a* is allelic to *eam8*. Like the 84 *mat-a* mutants, *eam8*.q, *eam8*.r, *eam8*.s, *eam8*.u and *eam8*.v are induced mutants, whereas *eam8*.k and *eam8*.w occur naturally in the cultivars Kinai5 and Early Russian, respectively. Mutant line *mat-a*.8, under the name Mari, was the very first early barley mutant to be released as a commercial cultivar. In this work we show that *Mat-a* is an ortholog of the Arabidopsis circadian clock gene *ELF3*. Wheat has an Earliness per se (Eps) locus which has been mapped between markers VacA and Smp. We were able to identify the VacA marker as orthologous to marker ABG055 in barley, and VacA and Smp as markers Sb09g030620.1 and Sb09g030810.2, respectively, in sorghum. Between the markers in sorghum we identified a gene orthologous to *A. thaliana Early Flowering 3* (*ELF3*). We have identified several unique mutations in barley *ELF3* correlated with an early flowering phenotype.

A novel QTL for type II *Fusarium* head blight resistance mapped on chromosome 7D of wheat.

M. Cativelli[1], M.L. Appendino[2], S. Lewis[1]

[1]Instituto de Recursos Biológicos, CIRN, INTA, Hurlingham (1686), Buenos Aires, Argentina
[2]Cátedra de Genética, Facultad de Agronomía, Universidad Nacional de Buenos (C1417DSE), Buenos Aires, Argentina

Fusarium head blight (FHB) is a common fungal wheat disease associated with yield and quality losses and mycotoxin grain contamination. Host-plant resistance is the most practical, effective, and economic way of FHB control, but new sources of resistance are limited. The moderately resistant spring wheat cultivar, Catbird, released by CIMMYT, may provide a source of resistance different from Sumai 3, the most heavy reliance source worldwide, due to its unrelated pedigree. The objective of this study was to identify QTLs associated with type II FHB resistance in cv. Catbird. A population of 102 double haploid lines derived from a cross between Catbird and Milan (a FHB-susceptible cultivar) was genotyped with 190 microsatellites markers and assessed by single floret inoculation method in a greenhouse during 2007 and 2008. Three QTLs were detected. They were mapped on chromosomes 3BS, 5DL and 7DS and named *Qfhb.3b*, *Qfhb.5d* and *Qfhb.7d*, respectively. All QTLs were derived from the resistant parent Catbird and were stable across years with the exception of *Qfhb.5d*. The QTL *Qfhb.7d* showed the largest effect explaining on average 20 % of the phenotypic variation. The *Qfhb.7d* peak was mapped in the centromeric region of 7DS between markers *Xbarc128* and *Xbarc214*. *Qfhb.3b* was a minor QTL mapped between markers *Xbarc133* and *Xgwm493* in a similar position to that of *Fhb1* in Sumai3 and derivates and explained 8.2 % of the phenotypic variation. *Qfhb.5d* was mapped in the vicinity of *Vrn-D1* locus, explained 14 % of the total variation during 2008 and needs an appropriate validation because of its unstable expression during 2007. The presence in Catbird of a QTL on chromosome 7DS is the first report that involves this wheat chromosome with the resistance to *Fusarium*. Markers flanking *Qfhb.7d* would facilitate its introgression in breeding programs. In addition, the pyramiding of *Qfhb.7d* with those QTLs from Sumai 3 and other sources could enhance the level of FHB resistance in wheat.

QTL study for salinity tolerance in Australian bread wheat

N. Shamaya, M. Tester, Y. Shavrukov, P. Langridge

[1]Australian Centre for Plant Functional Genomics, University of Adelaide, Urrbrae, SA 5064, Australia

One of the major limitations to agricultural production is soil salinity in arid and semi-arid regions of the world, both in irrigated and dryland agriculture. Increasing salinity tolerance of wheat is a target for many wheat breeding programmes. This requires an understanding of the inheritance of salinity tolerance traits. In the present study, a population of recombinant inbred lines (RILs) derived from a cross between bread wheat (*Triticum aestivum* L.) cultivars Gladius and Drysdale, was used to study Na^+ accumulation in leaves as a major trait associated with salinity tolerance. Two growth conditions were used: a supported hydroponics system and pots with soil. In an initial experiment, 200 RILs and their parents were grown in supported hydroponics, and 100 mM of NaCl was applied stepwise at the appearance of the third leaf. Seedlings were phenotyped for Na^+ accumulation in the fourth leaf and results were introgressed into a molecular map for QTL analysis. A negative correlation was observed between Na^+ accumulation in leaves and salt tolerance index, confirming that Na^+ accumulation is a one component of salinity tolerance in these RILs. The initial result showed that a suggestive novel QTL for Na^+ accumulation, which overlapped with a suggestive QTL for salinity tolerance index, was detected on chromosome 3B. However, the map resolution of this chromosome is poor (only 6 markers mapped to chromosome 3B) which is limiting the ability to detect significant QTL. 100 lines of 200 RIL were selected and are currently being genotyped using SNP markers. This will improve the linkage map of this mapping population. Further screening has also been conducted in pots and the association between different phenotypic traits including: salinity tolerance index, Na^+ and K^+ accumulation, Na/K ratio, osmotic stress tolerance have been studied. Further statistical analysis will be conducted to investigate phenotypic trait data and to identify QTL associated with these traits.

Introgressing novel salinity tolerance traits into commercial durum wheat

N. Shamaya, Y. Shavrukov, P. Langridge, M. Tester

Australian Centre for Plant Functional Genomics, University of Adelaide, Urrbrae, SA 5064, Australia

Durum wheat is a commercial cereal crop grown throughout the world, both on irrigated and rain-fed land. Much of the world's arable land, however, is affected by salinity, and this is a major limitation to durum wheat production. Increasing salinity tolerance in durum wheat cultivars is a feasible approach to tackling salinity. Landrace species related to durum wheat may be a source of novel salt tolerance traits for introgression into cultivated durum wheats. Previously, during massive screening, two Afghani landraces (lines 740 and 752) were identified as low Na^+ accumulators that is associated with salinity tolerance, with approximately half the accumulation of an Australian modern durum wheat cultivar, Jandaroi. In the present study, lines 740 and 752 were crossed with Jandaroi and the two F_2 progenies were screened for salinity tolerance based on the Na^+ exclusion phenotype. The aim of this study is to introgress a novel Na^+ exclusion trait from the landrace lines into Jandaroi and to identify the gene(s) controlling this trait using bulk segregant analysis. Plants were grown in supported hydroponics, with 100 mM of NaCl applied when the third leaf first appeared. F_2 plants in both progenies were genotyped using *DArT* markers. A putative association between four groups of *DArT* markers and Na^+ accumulation in two F_2 populations were identified associated with low Na^+ accumulation. 140 SSR markers that flanked to these groups of *DArT* markers were screened across the parents. 16 of the 140 markers showed polymorphism between the parents. The putative polymorphism markers will be screened between low Na^+ bulk and high Na^+ bulk. The marker(s) that show high association with Na^+ accumulation will be used to screen the entire population.

Characterization and mapping of durum wheat lipoxygenases

I. Garbus[1], D. Soresi[1], J. Romero[1], A. Carrera[2], V. Echenique[1, 2]

[1]CERZOS-CONICET. Camino "La Carrindanga" Km 7. B8000FWB Bahía Blanca – Argentina
[2]Departamento de Agronomía, Universidad Nacional del Sur, San Andrés 670. Bahía Blanca - Argentina

Durum wheat (*Triticum turgidum* L. ssp. durum, genomes AABB) constitutes the cereal of preference for semolina and pasta production. A bright yellow color is an important quality criterion for pasta making. Yellow color depends on the amount of carotenoid pigments in grain, which is the result of the balance between pigment synthesis and degradation, mainly by lipoxygenases (LPX). The number and location of lipoxygenases genes in durum wheat (*Triticum turgidum* L. ssp. *durum)* genome is not completely understood. Our laboratory recently reported the existence of a duplication at the *Lpx-B1* locus (*Lpx-B1.1* and *Lpx-B1.2*), mapped on chromosome 4B, and associated the deletion of the Lpx-B1.1 copy, with a reduction in lipoxygenase activity. A second lipoxygenase locus, Lpx-A3, associated with better semolina and pasta color was mapped on the homoeologous region on chromosome 4A. Here we focused on the physical organization of *Lpx* loci in chromosome 4A, where was identified a partially deleted copy of *Lpx*-1, called *Lpx-A1_like*. This copy colocalized within a 42 kbp region with Lpx-A3. The analysis of the sequences amplified with *Lpx-A1_like* based specific primers from the variety Kofa and the line UC1113 revealed the presence of two different deleted copies in UC1113 whereas only one was detected in Kofa, as was reported for *Lpx-B1*. This polymorphism was amplified in the available RIL population UC1113 x Kofa, to further map *Lpx-A1_like* in chromosome 4A short arm, between the microsatellite gwm192b and the locus Lpx-A3, thus confirming that in both genomes these two genes are close to each other. Finally, the *Lpx1* locus was amplified from the variety Urartu, the ancestral donor of durum wheat genome A, being identified a unique functional copy comprised between exon 2 and exon 8. This finding suggests that both the deletion and the duplication identified in durum wheat genome A were not inherited from the ancestor. The knowledge of the physical location and structure of *Lp x* genes is important to understand the on evolution of this family but also has practical implications for breeding since closely linked genes are difficult to separate by recombination.

A gene coding for a heavy-metal associated protein, *HMA-B1,* is associated with grain cadmium concentration in durum wheat.

C. Pozniak[1], K. Wiebe[1], N. Harris[2], J. Clarke[1], F. Clarke[3], A. Sharpe[4], G. Taylor[2]

[1]Crop Development Centre and Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada,
[2]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.
[3]Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1030, Swift Current, SK S9H 3X2, Canada
[4]Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Saskatchewan, Canada

Cadmium (Cd) is an environmental hazard that can have negative effects on human health. The dominant source of human exposure to environmental Cd is through contaminated food. Among cereals, some durum wheat cultivars have a genetic propensity to accumulate Cd in grain to levels exceeding proposed international limits of 100 ppb. A single major gene on chromosome 5B (*Cdu-B1*) regulates grain Cd levels but the genetic factor(s) conferring the low Cd phenotype are currently not known. Sequence analysis of bacterial artificial chromosomes spanning *Cdu-B1* revealed the presence of a gene coding for a heavy metal associated protein (*HMA*) which we have designated as *HMA-B1*. Allelic variation was assessed in lines isogenic for *Cdu-B1,* and a 17 bp insertion in the first predicted exon was identified in the high Cd accumulating line. Using a fine-mapping population of just over 4000 F_2 plants, the insertion co-segregated with phenotypic variation in grain Cd concentration. Based on the predicted protein sequence, the 17 bp insertion generates a premature stop codon, and thus a non-functional *HMA-B1* protein in high accumulators. We identified several knock-out mutations of *HMA-B1* from a TILLING population of CDC Verona, a low cadmium accumulating variety, and these are currently being assessed for grain Cd concentration. A co-dominant marker was developed using primers that flank the insertion, which we assessed in a global collection of over 100 durum wheat cultivars and breeding lines. No recombinations between the marker and grain Cd phenotype were detected in this collection. Given these data, *HMA-B1* is a strong candidate gene for *Cdu-B1* in durum wheat and is currently the target of detailed functional analysis.

QTL detection for water soluble arabinoxylan content and other grain quality traits in a hard hard wheat cross

P. Sharp, M. Turner, N. Howes, S. Begum

Plant Breeding Institute, University of Sydney, Cobbitty NSW 2570, Australia

Production of wheat with good physical, chemical characteristics of grain and flour quality is a desirable outcome for wheat breeding programs around the world. The first objective of this thesis was to analyze a doubled haploid (DH) population derived from hard wheat parents displaying similarity in phenotypes for the traits of interest. The F1 of the cross Goldmark x DM5637*B8 was used to produce a DH population by the wheat x maize system. The second objective was to map QTLs for multiple traits in this population. The traits included grain yield (GY), test weight (TW), thousand kernel weight (TKW), grain protein (GP), grain hardness (GH), flour yield (FY), water soluble arabinoxylans (WS-AX), water absorption (WA), dough development time (DDT) and dough breakdown (DB). The phenotypic data were analyzed by ASReml, a mixed model package. A total of 57 QTLs (including suggestive, significant, highly significant) covering 18 chromosomes were identified. There were 6 QTLs for WS-AX, 7 for GY, 7 for TW, 6 for TKW 6 for GP, 5 for WA, 5 for DDT, 4 for DB, 8 for FY and 5 for GH. This represents the first successful genetic analysis of WS-AX. The third objective was to investigate the genetic basis of the phenotypic correlation between important traits. The strong positive phenotypic relationship between WA and WS-AX were confirmed by co-locations of QTLs for these traits on chromosomes chromosome 3A and 5A. Independent QTLs were identified for the contrasting traits FY and WA and GY and GP, and this means that useful simultaneous improvement in these pair of traits will be possible.

High density mapping of wheat *QPm.tut-4A* gene region toward the gene cloning

M. Valárik[1], M. Kladivová[1], B. Klocová[1] D. Posti[2], I. Jakobson[2], H. Peusha[2], K. Järve[2], L. Timofejeva[2], Y. Hu[3], Y. Zhang[3], F.M. You[3], M.-C. Luo[3], A. Kumar[4], S.F. Kianian[4], H. Šimková[1], J. Šafář[1], J. Doležel[1]

[1]Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Sokolovská 6, CZ-77200 Olomouc, Czech Republic
[2]Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, Tallinn 19086, Estonia
[3]Department of Plant Sciences, University of California, Davis, CA 95616, USA
[4]Department of Plant Sciences, North Dakota State University, Fargo, Loftsgard Hall 470G, ND 58108, USA

Powdery mildew is one of major wheat pests causing substantial losses in grain yield and quality. The *QPm-tut-4A* gene conferring race nonspecific resistance to powdery mildew in both seedling and adult stage was recently introgressed to hexaploid wheat cv. Tähti from tetraploid wheat *Triticum militinae*. In the original mapping population derived from a cross of Tähti and resistant introgression line 8.1, the gene was mapped to distal part of chromosome arm 4AL in a region of approximately 10 cM delimited by markers *wmc232* and *wmc313*. The region comprises 12 additional markers. However, the order of markers could not be resolved using 2400 gametes. A new mapping population from a cross of the resistant line 8.1 and susceptible cv. Chinese Spring was created. In the mapping population, recombination was observed in the *QPm.tut-4A* region and the gene was delimited to a 1.8 cM region. Physical map of the 4AL chromosomal arm was constructed and screened with markers in the *QPm.tut-4A* region. The screening yielded three contigs with a total size of 3Mb and 296 BAC clones. Ends of 196 BACs were sequenced and 46% of them were suitable for marker development. Three of the BAC-end sequences shared homology with rice genes located on chromosome 6 and anchored the *QPm.tut-4A* locus to a collinear region in rice. A RIL F_8 genetic map of *T. monococcum* was saturated with 627 markers. Two markers designed based on a collinear rice gene OsJ_19832 were mapped to *T. monococcum* chromosome $7A^m$ and to the collinear region of chromosome $6A^m$. Flanking markers will be used to saturate the *QPm.tut-4A* region. To maximize the mapping resolution, a panel of 500 radiation hybrid lines was created from a resistant 4AL telosomic line carrying the *T. militinae QPm.tut-4A* region. The lines will be used to order markers in the *QPm.tut-4A* region and identify locus carrying the gene. This work has been supported by the Czech Science Foundation (521/08/1629), by the Ministry of Education, Youth and Sports of the Czech Republic and the European Regional Development Fund (Operational Programme No. CZ.1.05/2.1.00/01.0007), and by the Estonian Ministry of Agriculture.

Yr48, a new and validated source of partial resistance to broadly-virulent races of wheat stripe rust

I. Lowe[1], L Jankuloski[1, 2], J. Hegarty[1], S. Chao[3], X. Chen[4], D. See[4], J. Dubcovsky[1,*]

[1]Department of Plant Sciences, University of California – Davis; CA
[2]Department of Genetics and Plant Breeding, Faculty of Agricultural Sciences and Food; Skopje, Macedonia
[3]USDA-ARS Cereal Crops Research Unit; ND 4 USDA-ARS Wheat Genetics, Quality, Physiology and Disease Research Unit; WA
*E-mail: jdubcovsky@ucdavis.edu

A hexaploid wheat mapping population was evaluated for its response to current California races of stripe rust in replicated field trials over four seasons (2007-2010) in the northern Sacramento Valley. A genetic map was constructed consisting of 1,494 polymorphic probes mapped to 558 unique loci; and QTL analysis revealed the presence of four stripe rust resistance QTL, two from UC1110 (on chromosomes 3BS and 2BS) and two from PI610750 (5AL and 2AS). The two QTL of largest effects (3BS and 5AL) were validated in independent F2 populations and their intervals narrowed to 2.5 cM and 5.3 cM, respectively. The 3BS QTL (QYr-ucw.3BS) was shown by allelism tests to carry a gene different from either the Yr30 or Yrns-B1. Mapped position also suggests that it carries a gene different from YrRub. An additional season of data (2011) revealed the resistance conferred by QYr-ucw.3BS to be race-specific. The 5AL QTL carries a previously unreported partial stripe rust resistance gene, here designated Yr48. Yr48 exhibits additive gene action and as yet shows no race specificity. Further dissection of the Yr48 region reduced its interval to 0.11 cM, small enough to begin the construction of a physical map of the region.

Three population approach for marker identification and simultaneous validation of non-hypersensitive adult plant stripe rust resistance in wheat

R. Tiwari[1], S.Pawar[1], M.S. Saharan[1], R. Singh[1], B. Keller[2], V. Tiwari[1], I. Sharma[1]

[1]Directorate of Wheat Research, Karnal- 132001, Haryana,
[2]Institute of Plant Biology, University of Zürich, CH 8008 Zürich, Switzerland

India had a record wheat production of 84.27 million tons during 2010-11 crop season, inspite of the high level of stripe rust inoculums built up in India's northern wheat growing region. Majority of the popular cultivars of Hill Zone and many from the North Western Plains Zone succumbed to stripe rust. So there is an urgent need to identify molecular markers linked to the genes for stripe rust resistance, for accomplishing marker assisted pyramiding. In an effort using bulk segregant approach to identify molecular markers for stripe rust resistance from Cappelle Desprez, putative marker on 2D chromosome has been identified. Recombinant inbred line population was evaluated for stripe rust under artificially inoculated conditions in poly house at Karnal with predominant pathotype 78S84. Results indicated presence of more than one gene imparting stripe rust resistance in Cappelle Desprez, effective against pathotype 78S84. Therefore, for molecular mapping of the resistance, a three population approach involving a population developed out of Cappelle Desprez / Cappelle Desprez (Mara-2D), Cappelle Desprez / PBW 343 and Cappelle Desprez (Mara-2D) / PBW 343 has been applied. This approach would be helpful in identification as well as simultaneous validation of the markers from 2D and the additional gene from other part of the genome. Detailed results would be presented during the meeting.

Use of Consensus Maps to Differentiate QTL for Leaf and Stripe Rust Resistance

G.M. Rosewarne[1], R.P. Singh[2], S.A. Herrera-Foessel[2], J. Huerta-Espino[3].

[1]CIMMYT 4 Shizishan Rd, Jinjiang, Chengdu, Sichuan Province, P.R. China 610066
[2]CIMMYT Apdo. Postal 6-641, 06600, Mexico D.F., Mexico
[3]Campo Experimental Valle de Mexico-INIFAP, Apartado Postal 10, 56230, Chapingo, Edo. de Mexico, Mexico

Over the past ten years there have been at least 15 and 25 publications on QTL associated with leaf rust and stripe rust resistance, respectively. Each publication generally reports multiple resistance loci, resulting in a number of QTL from different sources on nearly every chromosome. To complicate the issue different marker platforms such as RFLP, SSR, AFLP, CAPS, RAPD, DArT, STS, RGAP and SNP were used in these studies. This makes it very difficult to identify which loci are likely to be novel and available for recombination in breeding programs. For example, recent work on the Avocet × Pastor RIL population identified the *Lr46/Yr29* locus on 1BL and separate loci for leaf rust and stripe rust severity on the same chromosome. There were seven other reports in the literature identifying 1B QTL for these rusts. Four of these were identified as the *Lr46/Yr29* locus with the other three reports potentially being different loci. Consensus maps of all flanking markers were investigated and the QTL formed two major clusters. We were able to locate the two Pastor QTL for leaf and stripe rust severity on a 1BS section that grouped with leaf rust QTL identified in the winter wheat "Forno" (Messmer et al. 2000; Schnurbusch et al. 2004). We also identified the *Lr46/Yr29* locus of Pastor with numerous flanking markers from other studies on 1BL (Lillemo et al 2008; Suenaga et al. 2003; William et al. 2006; Melichar et al. 2008). A third region associated with a High Temperature Adult Plant (HTAP) resistance gene (Lin and Chen 2009) was not differentiated from the *Lr46/Yr29* locus as the Resistance Gene Analogue Polymorphic markers used to define the HTAP QTL had not been used in any *Lr46/Yr29* studies. However, we have shown that linked SSR markers place the HTAP locus between the two abovementioned clusters. Data is presented on a number of chromosomes where multiple QTLs have been identified as a means to separate the loci and give clues as to where allele testing would be needed to further discriminate loci.

QTL mapping of durable adult plant stem rust resistance to Ug99 in CIMMYT wheat lines- Kingbird, Kiritati and Juchi

S. Bhavani[1*], R. P. Singh[2], O. Argillier[3], J. Huerta-Espino[4], S. Singh[2], P. Njau[5]

[1]CIMMYT, Nairobi, Kenya;
[2]CIMMYT, Apdo. Postal 6-641, 06600, Mexico, DF, Mexico
[3]Syngenta Seeds S.A.S Ferme de Moyencourt 78910 Orgerus, France
[4]INIFAP-CEVAMEX, Apdo. Postal 10, 56230, Chapingo, Mexico;
[5]Kenyan Agricultural Research Institute, (KARI)- Njoro, P.O. Njoro, Kenya
[*]E-mail: s.bhavani@cgiar.org

The identification of new sources of resistance to TTKSK and its related races is necessary to enable the development and deployment of resistant varieties that have race-nonspecific, adult plant resistance (APR) conferred by multiple minor, slow rusting genes. Wheat lines 'Kingbird, 'Kiritati' and 'Juchi' showed high levels of APR to Ug99 races of stem rust fungus when tested in Kenya. The F5 and F6 generation recombinant inbred line (RIL) populations developed from the crosses of moderately susceptible 'PBW343' with the three resistant parents were used in mapping. Field phenotyping of the parents and RILs were conducted at Njoro, Kenya for at least two years with Ug99+*Sr24* (TTKST) race under high stem rust pressures. The continuous variation of APR in each RIL population and genetic analyses indicated quantitative nature of resistance that was likely governed by 3 or 4 minor genes. Single and joint year analyses by Inclusive Composite Interval Mapping (ICIM) using informative DArT and/or SSR markers identified consistent APR QTLs on chromosomes 1A, 3BS, 5BL, 7A and 7DS in Kingbird; 2D, 3BS, 5BL and 7DS in Kiritati and 2B, 3BS, 4A, 5BL and 6B in Juchi. QTLs on each genomic regions explained 10- 46% of the phenotypic variation for APR. Pseudo-black chaff phenotype associated with APR gene *Sr2* on chromosome 3BS in all six resistant parents and identification of an APR QTL in the same region in all mapping populations confirmed the role of *Sr2* in reducing stem rust severity. The QTL on chromosome 7DS in Kingbird was in the same region where pleiotropic APR gene *Lr34/Yr18/Pm38* is located. Further studies are underway to saturate the genomic regions harboring new APR QTLs with additional molecular markers.

QTLs mapping of durable adult plant resistance to stem rust Ug99 of Wheat in population PBW343/MUU

S. Singh[1], R.P. Singh1, S. Bhavani[3], J. Huerta-Espino[2], K.S. Gill[4], E.E. Lopez[1]

[1]CIMMYT, Apdo. Postal 6-641, 06600, Mexico DF, Mexico
[2]INIFAP-CEVAMEX, Apdo. Postal 10, 56230, Chapingo, Mexico
[3]CIMMYT, Nairobi, Kenya
[4]WSU, Pullman, USA

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is potentially one of the most serious diseases of wheat in several wheat growing regions of the globe. Genetic enhancement for the disease involves the quantitative nature of inheritance, which reflects the additive effects of several genetic loci all over the genome. QTL mapping can offer paved initial points for identifying such candidate genes/ alleles, which can further be used for the introgression-breeding program. The advanced line MUU showed elevated levels of adult plant resistance to stem rust when tested in Kenya for last five years. Recombinant inbred line (RIL) population developed from a cross of resistant parent MUU and susceptible parent PBW343 and field tested in two crop seasons 2009 and 2010 against stem rust pathogen. The distribution of the disease severity in the RIL population varied from highly resistant (less than 10% severity) to susceptible (more than 80% severity) confirming the quantitative nature of the effects of the QTL for resistance. A genetic map of wheat with 492 the Diversity Array Technology (DArT) and 74 simple sequence repeat (SSR) markers was constructed using a population of inbred line (RIL) from a cross between the PBW343 and MUU. Linkage groups were assigned to wheat chromosomes using available map locations of SSR markers as reference points. Inclusive Composite Interval Mapping (ICIM) identified genomic regions associated with low disease severity on chromosomes 2B, 3BS, and 5BL. These genomic regions explained 5- 36% of the phenotypic variation in adult plant stem rust reaction. The QTL identified on chromosome 3BS suggests the contribution of Sr2 in reducing the disease severity. The QTL on chromosome 5BL and 2B explained 5 - 8% of phenotypic variance and was reliable in both the years. Enrichment of chromosome 5BL with more markers will be discussed. The markers within these QTL regions present an opportunity for marker-assisted selection for stem rust in wheat breeding programs.

Genome-wide association mapping for stem rust resistance at the adult stage in elite durum wheat germplasm

T. Letta[1, 4], M. Maccaferri[1], A. Badebo[2], K. Ammar[3], M.C. Sanguineti[1], J. Crossa[3], P. Oliveira[5], Y. Jin[6], R. Bovina[1], R.Tuberosa[1]

[1]Department of Agroenvironmental Science and Technology (DiSTA), University of Bologna, Viale Fanin 44, 40127 Bologna, Italy
[2]Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia
[3]CIMMYT, Int. Apdo Postal 6-641, Mexico D.F., Mexico 06600
[4]Sinana Agricultural Research Center, Bale-Robe, Ethiopia
[5]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA
[6]USDA-ARS, Cereal Disease Laboratory, St.Paul, MN 51108, USA

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is a highly destructive fungal disease of wheat. One of the best approaches to alleviate this threat is to identify and characterize sources of resistance within the available wheat breeding materials and commercial cultivars. To this end, a panel of 183 elite durum cultivars suitable for association mapping (Maccaferri et al., 2005 Plant Genetic Resources, 4:79-85) was characterized for stem rust response for four consecutive seasons (off- and main-seasons) in 2009 and 2010 under field condition, at the Debre Zeit Research Center, Ethiopia, a reputed 'hotspot' site for stem rust epidemics on tetraploid wheats. The panel was artificially inoculated using isolates comprising of Ug99 and a local mixture of urediniospores of durum-specific races prevalent in Ethiopia. Maximum Disease Severity Scores (DSS%) were converted into Coefficient of Infection (CI) for carrying out AM analysis. On average, CI in the off-seasons was markedly higher than that registered in the main-seasons. About 13 lines had CI values < 10 in off-seasons while more than 30 lines had CI value < 10 in main seasons. The phenotypic distribution based on CI data indicates that the overall genetics of resistance in the panel is complex. The molecular profiles of the panel obtained using 323 SSR and 900 DArT markers were subjected to association mapping analysis using mixed linear model with population structure and familial relationships controlled by Q matrix and by kinship matrix, respectively. Several chromosome regions putatively involved in stem rust response were identified across seasons. The four regions with the largest effect were mapped on chrs. 1B, 5A, 6A (coincident with the chr. position of *Sr13*) and 7B and showed R^2 values ranging from 2.3-5.6, 2.5-5.7, 1.5-11.3 and 1.5-5.7%, respectively. Significant associations were also detected in other chr. regions not known to harbor stem rust resistance genes. Our study has identified a number of novel regions for resistance to Ug99 and other Ethiopian races that have the potential to contribute via marker-assisted selection to the improvement of resistance to stem rust in durum wheat.

Association mapping for stem rust resistance at seedling stage in durum wheat

T. Letta[1,6], P. Oliveira[2], Y. Jin[3], M. Maccaferri[1], A. Badebo[4], M.C. Sanguineti[1], K. Ammar[3], R. Tuberosa[1]

[1]Department of Agroenvironmental Science and Technology (DiSTA), University of Bologna, Viale Fanin 44, 40127 Bologna, Italy
[2]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA
[3]USDA-ARS, Cereal Disease Laboratory, St.Paul, MN 51108, USA
[4]Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia
[5]CIMMYT, Int. Apdo Postal 6-641, Mexico D.F., Mexico 06600
[6]Sinana Agricultural Research Center, Bale-Robe, Ethiopia

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* is one of the most destructive diseases of durum and bread wheat. Races that recently emerged in East Africa (TTKSK; Ug99 and its derivatives) possess broad virulence to wheat cultivars worldwide. A panel of 183 elite durum cultivars suitable for association mapping (Maccaferri et al., 2005 Plant Genetic Resources, 4:79-85) was evaluated for stem rust response at seedling stage. The panel was challenged with three *Pgt* races with broad virulence viz. TTKSK (Ug99), TRTTF and TTTTF. About 43% of lines showed resistance to the three races (with disease scores ≤ 2 based on 0 -4 scale). The portion of lines resistant to race TTKSK, TRTTF and TTTTF was 50%, 78% and 60%, respectively. The molecular genotypes of the lines (obtained with 323 SSR and 900 DArT markers) were subjected to association mapping analysis employing Mixed Linear Model using Tassel programme 2.1. Several chromosome regions putatively involved in race-specific stem rust response were identified. The largest effect on resistance to TTKSK and TTTTF races was detected on chr. 6A (haplotype tagged by *barc104*, *CD 926040* and *BE403950*) in a region coincident with the previously identified resistance gene *Sr13*. Additionally, a sizeable effect on the resistance to both races was detected on chr. 7B (*gwm517* and *gwm1070*). Significant effects for resistance to race TRTTF were detected on chrs. 2A (*gwm636* and *PPD-A1*), 2B (*gwm47* and *gwm846*) and 7A (*gwm735*, *gwm344* and *cfa2257*). Thirteen common DArT markers showed significant effect for resistance to three races and several race-specific associations were also detected. These results suggest that the elite durum genepool surveyed in our study has several loci for resistance to Ug99 and other important stem rust races.

Association mapping of leaf stripe resistance in a European spring barley germplasm collection

A. Tondelli[1], N. Faccini[1], A. Marrano[2], A. Blanco[2], J. Comadran[3], R. Waugh[3], A. Flavell[4], L. Cattivelli[1], G. Valè[1]

[1]CRA – Genomics Research Centre, Via San Protaso 302, 29017 Fiorenzuola d'Arda, Italy
[2]University of Bari, via Amendola, 165/A, 70126 Bari, Italy
[3]Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK
[4]University of Dundee at SCRI, Invergowrie, Dundee DD2 5DA, UK

Within the ERA-PG funded project ExBarDiv (Genomics-assisted analysis and exploitation of barley diversity) three different barley populations - namely cultivar, landrace and wild (*Hordeum spontaneum*) germplasm collections - have been assembled in order to test the efficiency of an incremental association mapping approach for identifying new useful gene alleles. As a first step, here we report the evaluation of 230 spring 2-rowed barley cultivars for their resistance to leaf stripe, a widespread seed-borne disease caused by the fungal pathogen *Pyrenophora graminea*. For each line, sixty seeds have been surface-sterilized and incubated in three Petri dishes between two Potato Dextrose Agar layers colonized by an actively growing mycelium of the *P. graminea* isolate Dg5. After 20 days of incubation in the dark at 6 °C, the emerged seedlings have been transplanted to pots and grown in the greenhouse (20°C, 14 h light and 12°C, 10 h night). The whole experiment has been repeated twice, and resistance has been assessed as the percentage of plants showing leaf stripes symptoms. The same barley collection has been genotyped with ~7000 gene-based SNPs using the Illumina™ iSelect high throughput marker technology. The use of a large diverse germplasm population and high numbers of markers has allowed us to investigate: i) trends in the patterning of genetic diversity in European cultivar barley in time and space; ii) the utility of a diverse cultivar population for discovering useful associations between genetically mapped markers and important traits such as resistance to fungal pathogens.

Genome wide association studies of four fungal disease resistance in synthetic hexaploid wheats.

F.C. Ogbonnaya[1§], M.A. Mulki [1], R. Trethowan[2], J. Shepperd[3], H.S. Bariana[2], F. Dreccer[4], M. Imtiaz[1], G. Ye[5], L.C. Emebiri[6], D.B. Moody[7], O. Abdalla[1,] M. Baum[1], E.S. Lagudah[8]

[1]International Center for Agricultural Research in the Dry Areas (ICARDA), P. O. Box 5466, Aleppo, Syria
[2]University of Sydney Plant Breeding Institute-Cobbitty, PMB11, Camden, New South Wales 2570, Australia
[3]Department of Primary Industries and Fisheries, Agency for Food and Fibre Sciences, Toowoomba, QLD, Australia
[4]CSIRO Plant Industry Cooper Laboratory, PO Box 863, University of Queensland, Warrego Highway, Gatton, QLD 4343, Australia
[5]International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines
[6]EH Graham Centre for Agricultural Innovation, Pine Gully Road, Wagga Wagga, NSW 2650, Australia
[7]InterGrain Pty Ltd, 3 Baron-Hay Court, Kensington 6151, Western Australia
[8]CSIRO Plant Industry, GPO Box 1600, Canberra, Australian Capital Territory, 2601, Australia

Fungal diseases in wheat are responsible for substantial economic losses globally and represent major risks with food security implications in many developing countries where wheat is grown. With recent advances in gene technology, and increasing impetus to exploit natural diversity, we employed genome-wide association scan to identify genomic regions associated with quantitative trait loci for resistance to fungal diseases in synthetic hexaploid wheat that are widely reported as sources of useful genes for common wheat improvement. Three hundred and thirty two synthetic hexaploids were evaluated for four fungal disease resistances – yellow leaf spot (YLS; *Pyrenophora tritici-repentis*), leaf rust [LR; *Puccinia triticina* (formerly *Puccinia recondita* f. sp. *tritici*)], stem rust (SR; *Puccinia graminis* f. sp. *tritici*), and stripe rust (YR; *Puccinia striiformis* f. sp. *tritici*). These were genotyped with DArT, including SSR and STS markers previously reported to be linked to resistance to the fungal pathogens. Population structure was evaluated by principal component analysis and Structure, which classified the synthetic hexaploid wheat into seven subgroups. Genome wide association study (GWAS) was carried out using general linear model with Q-matrix and the mixed linear model with K-matrix. Several DArT makers were associated with resistance to the fungal pathogens singly or in combination at ($P \leq 0.05$). Of most significance is the identification of multiple loci that confer multi-pathogen resistance including a DArT marker on chromosome 5B that conferred resistance to YLS, Lr, Sr and Yr. Some of the identified loci were tied closely to previously identified genes while several new marker alleles were identified to be associated with unreported resistance genes amongst the fungal pathogens.

Identification of QTLs for resistance to septoria tritici blotch in durum wheat through association mapping

M. Maccaferri[1], K. Ammar[2], J. Crossa[2], M.S. Gharbi3, A. Massi[4], R. Talebi[5], T. Ghaffary Mahmod[5,6], G.H.J. Kema[6], S. Corneti[1], R. Tuberosa[1], M.C. Sanguineti[1]

[1]DISTA, Viale Fanin 44, University of Bologna, 40127 Bologna, Italy
[2]CIMMYT, Int. Apdo Postal 6-641, Mexico City, 06600 Mexico
[3]Laboratory of Cereal Genetics, INRAT/IRESA, Hedi Karray street, 2080 Ariana, Tunisia
[4]Società Produttori Sementi Bologna (PSB), 40050 Argelato (BO), Italy
[5]Plant Research International B.V., 6700 AA Wageningen, The Netherlands
[6]Safiabad Agricultural Research Center, P.O. Box 333, Dezful, Iran

Durum wheat production in the Mediterranean basin is plagued by a range of biotic stresses. Among those, septoria tritici blotch that is caused by the fungus *Mycosphaerella graminicola* has become an important disease. The high genome plasticity of the pathogen and its specialization features (differential pathogenicity towards durum and bread wheat) hinder the identification and exploitation of resistance genes across diverse growing areas. The genetic variation of the response to *M. graminicola* and the chromosomal location of resistance factors were studied in a durum wheat panel of 183 accessions of diverse origin suitable for association mapping (Maccaferri et al. 2006, Plant Genetics Resources 4: 79-85). The panel was evaluated for two consecutive years (2008 and 2009) in three environments: Tunisia (Beja), Mexico (Toluca) and Italy (Argelato and Ferrara). The accessions were then inoculated under controlled conditions with ten *M. graminicola* isolates from durum wheat collected over a range of Mediterranean countries. The germplasm collection has been genotyped with approximately 300 SSR markers of known map position and about 900 durum wheat DArT markers. Based on the field and the seedling inoculation assay data (percentages necrosis and pycnidia on primary leaves), significant marker-trait associations pointing to specific chromosome regions have been obtained. A preliminary analysis highlighted some chromosome regions consistently involved in resistance to septoria tritici blotch in bread wheat, particularly in chromosomes 1BL, 2AL and 4AL that accounted for a sizeable portion of phenotypic variation among accessions. The detailed results will be presented and discussed.

Association of twelve candidate genes with frost tolerance in rye in controlled, semi-controlled and field phenotyping platforms

Y. Li[1], A. Böck[2], G. Haseneyer[1], V. Korzun[3], P. Wilde[3], C.C. Schön[1], D. Ankerst[2], E. Bauer[1]

[1]Technische Universität München, Center of Life and Food Sciences Weihenstephan, Plant Breeding, Freising, Germany
[2]Technische Universität München, Mathematical Statistics, Garching, Germany
[3]KWS LOCHOW GMBH, Bergen, Germany

Frost is an important abiotic stress that limits cereal production in the temperate zone. As the most frost tolerant small grain cereal, rye (*Secale cereale* L.) is an ideal cereal model for investigating the genetic basis of frost tolerance (FT), a complex trait with polygenic inheritance. Using 201 genotypes from five Eastern and Middle European winter rye populations, this study reports a multi-platform candidate gene-based association analysis in rye using 161 SNPs and nine Indels previously identified from twelve candidate genes with a putative role in the frost responsive network. The candidate genes comprised seven members of the *ScCbf* transcription factor family, as well as, *ScDreb2*, *ScDhn1*, *ScDhn3*, *ScIce2*, and *ScVrn1*. Intra-genic linkage disequilibrium (LD) decayed rapidly below $r^2 < 0.2$ within 400 bp on average. Using 37 SSR markers, population structure and kinship were determined resulting in a Q-matrix and a K-matrix for association analyses using linear mixed models. Statistically significant associations between FT and SNPs of nine candidate genes were identified. Two SNPs in *ScCbf15* and one in *ScCbf12*, all leading to amino acid exchanges, were significantly associated with FT over all three phenotyping platforms. Distribution of SNP effect sizes expressed as percentage of the genetic variance explained by individual SNPs was highly concentrated near 0 with a few SNPs obtaining large effects. Two-way epistasis was found between 14 pairs of candidate genes. Relatively low to medium empirical correlations of SNP-FT associations were observed across the three platforms underlining the need for multi-level experimentation for dissecting FT in rye. Our results demonstrated that the candidate gene-based association approach remains one of the most appropriate strategies for gene identification, given the huge genome size of rye (~8,100 Mb) and the rapid decline of LD. Validation of SNPs significantly associated with FT will be performed in future studies to determine the diagnostic value of markers for marker-assisted selection in rye breeding programs.

Linkage disequilibrium and population structure in tetraploid wheat

G. Laidò[1], F. Taranto[2], D. Marone[1], G. Mangini[2], A.M. Mastrangelo[1], L. Cattivelli[3], R. Papa[1], A. Blanco[2], P. De Vita[1].

[1]Cereal Research Centre (CRA-CER), Agricultural Research Council, S.S 16 km 675, 71122 Foggia, Italy
[2]Department of Environmental and Agro-Forestry Biology and Chemistry, University Aldo Moro, Via Amendola, 165/A, 70126 Bari, Italy
[3]Genomic Research Centre (CRA-GPG), Via San Protaso 302, 29017 Fiorenzuola d'Arda, Italy

Together with knowledge on population structure, a critical step for the planning of association and population genomics studies is the level of linkage disequilibrium (LD) that characterizes the specie and the population employed for the analysis. We have analyzed the population structure and the LD in a large collection of tetraploid wheats (*Triticum turgidum* subsp.) made of 128 accessions of durum wheat and 104 wild and domesticated accessions. All of the accessions were analyzed with 26 SSR and 821 DArT markers, most of which were genetically mapped. Our results partially reflect previous knowledge on population structure of tetraploid wheats, and they clearly show a sharp separation of durum wheat accessions from the rest of the naked and hulled tetraploid wheats. The population structure of durum wheat cultivars were in agreement the knowledge on the breeding history. Indeed, a strong correlation was found between the genetic structure of modern varieties and year of release. Landraces and wild accessions had a higher allelic diversity than modern durum wheat varieties for both genomes and all chromosomes in terms of total number of alleles and allelic richness. The wild accessions were characterized by very low levels of LD, while a higher LD value was observed for the subgroup containing the durum wheat genotypes (8.2, $P<0.001$). Wild and domesticated accessions represent a useful rich source of useful alleles for plant breeding and a powerful tool to detect and identify useful genes using association mapping and population genomics studies.

A consensus map for durum wheat as a framework for linkage, association mapping studies and SNP development

M. Maccaferri[1], A. Massi[2], F. Clarke[3], C. Pozniak[4], R. Knox[3], J. Clarke[4], H. Buerstmayr[5], M. Buerstmayr[5], A. Korol[6], T. Fahima[6], S. Xu[7], I. Karsai[8], G. Vida[8], M.C. Sanguineti[1], R. Tuberosa[1]

[1]Department of Agroenvironmental Science and Technology, University of Bologna, Viale Fanin 44, 40127 Bologna, Italy
[2]Società Produttori Sementi Bologna (PSB), Via Macero 1, 40050 Argelato (BO), Italy
[3]Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1030, Swift Current, SK S9H 3X2, Canada
[4]Crop Development Centre and Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada
[5]University of Natural Resources and Life Sciences, Department for Agrobiotechnology (IFA-Tulln) Institute for Biotechnology in Plant Production A-3430 Tulln Austria
[6]Department of Evolutionary and Environmental Biology, Institute of Evolution, Faculty of Science and Science Education, University of Haifa, Mt. Carmel, Haifa 31905, Israel
[7]Research Geneticist USDA/ARS Cereal Crops Research Unit, NCSL 1605 Albrecht Blvd. N., Fargo, 58102-2765 ND, USA
[8]Agricultural Research Institute of the Hungarian Academy of Science, H-2462 Martonvasar, Hungary

Although the world durum wheat production is estimated to be less than 10% of global wheat production, durum wheat is the main cereal crop in some regions of the Mediterranean Basin (Southern Europe, NorthAfrica and Western to Central Asia), North American Great Plains (USA and Canada) as well as the Southwestern part of USA, Mexico, Australia and India. The molecular tools necessary to identify the loci governing traits of interest are now available in most species. In hexaploid wheat, numerous SSRs were developed and used to construct linkage maps. Hence, genetic mapping and QTL analysis in durum wheat can be performed with molecular markers developed for the A and B genomes of hexaploid wheat. The SSR-based maps can be further saturated with the more recently developed high-throughput markers, such as those based on microarray-hybridization assays (DArT® markers), that have been specifically developed for durum wheat. Mapping populations and molecular linkage maps were recently developed in durum wheat, with the aim of mapping factors for resistance to various diseases and grain/semolina quality traits. This provides the opportunity for combining linkage mapping data from diverse materials and obtaining a consensus map specific for durum wheat that is potentially useful as a framework for: (i) more accurate marker choice in marker-assited selection programs, (ii) meta QTL analysis, (iii) placement of significant markers identified by association mapping, (iv) mapping novel SNP markers which are currently being developed in both common and durum wheat, and (v) studying the evolution of the A and B genomes of durum wheat relative to hexaploid wheat. We compiled mapping data from 19 mapping populations (i.e. four double haploid, seven recombinant inbred line and four backcross inbred line populations). The mapping populations were genotyped with SSR, AFLP and DArT® markers, and in total, ca. 7,500 molecular markers are available for consensus mapping. The data are being analyzed using two different consensus mapping approaches and results will be reported and discussed.

Development of a high-density consensus map in durum wheat

D. Marone[1], G. Laidò[1], A. Gadaleta[2], P. Colasuonno[2], A. Giancaspro[2], S. Giove[2], P. De Vita[1], A. Blanco[2], L. Cattivelli[1,3], R. Papa[1], A.M. Mastrangelo[1]

[1]CRA-CER Centre of Cereal Research, SS16 km675, 71122, Foggia, Italy
[2]Department of Agro-Foresty and Environmental Biology and Chemistry, University of Bari, Via Amendola, 165/A, 70126 Bari, Italy
[3]CRA-Genomic Reserach Centre Via S. Protaso, 302, 29017, Fiorenzuola d'Arda (PC), Italy

A well-saturated genetic map is an important tool for plant breeding and many technologies are available to increase the abundance of molecular markers suitable for genetic analysis, such as DArT markers. A consensus map of durum wheat (*Triticum turgidum* L. var. *durum*) was constructed based on segregation data from six mapping populations, including Creso x Pedroso, Ofanto x Cappelli, Cirillo x Neodur, Svevo x Ciccio, Messapia x MG4343 and Latino x Primadur. All listed genotypes are durum wheat varieties, except MG4343, which is an accession of *Triticum turgidum* var. *dicoccoides*. The composite map contained a total of 1916 markers, comprising SSR, EST-SSR, STS, TRAP, RFLP, morphological and biochemical markers. The total map length spanned 3021 cM spread over 25 linkage groups and showed a mean distance between neighbouring loci of 1.6 cM. Among all markers, 640 were common at least in two populations while 1276 were mapped in a single population. The comparison of marker order in the consensus and the individual maps, revealed a good co-linearity, except for few putative inversions in the frame of few cM. A small fraction (8%) of the markers deviated significantly from the expected Mendelian ratio; clusters of loci showing distorted segregation ($P<0.01$) were found on chromosomes 5A, 6A, 1B, 2B, 4B, 5B, 6B, and 7B. The analysis of map location of putative homoeologous loci suggests the occurence of several rearrangements in chromosomes 4A, 5A, 6A, and 7B. The putative translocation on chromosome 7B was previously described. This consensus map represents a very useful tool, providing a more complete coverage of the durum wheat genome, to facilitate genomic researches such as evolutionary studies, QTL fine mapping for map-based cloning, evaluation of the degree of linkage disequilibrium and association analysis of important agronomic traits.

Intra-specific map of durum wheat based on SSR and SNP markers

I. Yousefi Javan[1*], L. Mondini[1], M.N. Nachit[2], M.A. Pagnotta[1]

[1]Department of Science and Technologies for Agriculture, Forestry, Nature and Energy (DAFNE), Tuscia University, Viterbo, Italy
[2]International Centre for Agricultural Research in the Dry Areas (ICARDA), P.O.Box 5466, Aleppo, Syria
[*]E-mail: Iman.javan@unitus.it

Now a day several technologies are available to increase the abundance of DNA markers and to contribute in developing high resolution genetic maps suitable for genetic analysis. Among those simple sequence repeats (SSRs), highly dispersed in the genomes, were used for designing genetic maps and, in turn, estimating the genetic relationship between the A and B genome. A genetic linkage map of tetraploid wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.) will construct based on a intra specific cross between two recombinant inbred lines (RILs): one deriving from the cross of Jennah Khetifa x ChamI, the other from the cross [Omrabi x Dicoccoides] x Omrabi. The aim of this study is to expand the number of simple sequence repeat (SSR) and single-nucleotide polymorphism (SNP) markers on the wheat array that can be mapped in the wheat genome, and to determine their chromosomal location, and their position on the genetic structure. The 190 single-seed descent lines derived F8 RILs were analyzed with a total of 204 loci SSR and 20 loci SNP. After 7 generations of selfing looking at two genes in the F8, we will get a percentage of 98.45% homozygousity and 1.55% heterozygosity. It was also variable the number and location of SSR on chromosomes. The total number of markers and the density was higher in homoeologous groups 2 and 4 (with respect of a total of 73 and 35). Here are presented the ongoing results. After analyzing a total of 204 markers, clear results from204 of the parental screening, 113 markers SSR revealed polymorphism, while the rest were monomorphic. These markers were previously located on chromosome arms in ditelosomic and nullitetrasomiche aneuploid lines of *T .aestivum* cv. Chinese Spring. The SSR markers were distributed on all chromosomes of the two genomes in durum wheat (A and B), with the highest percentage of polymorphism in the A-genome (64%) comparing with the B-genome (57%). It was also variable, the percentage of polymorphism, both between chromosomes and between genomes of the seven homologues in this of tetraploid wheat species, The homology groups 1, 4 and 6 in the A-genome showed a highest level of polymorphism (54%, 21%, 38%, respectively). We are going to built a genetic linkage map. The total number of markers and its density is higher in homoeologous groups 2 and 4 (84 and 39 SSR). Each marker corresponds to one locus.

Keywords: Recombinant Inbred Line, LOX, Simple Sequence Repeats, A/B-genome

Posters

Session 3

Evaluation of wheat for *Fusarium* infection

Š. Sliková, V. Šudyová, E. Gregová, M. Hudcovicová

Plant Production Research Center Piešťany, Bratislavská cesta 122, SK-921 68, Piešťany, Slovak Republic

Fusarium head blight (FHB) is a disease occurring every year on wheat grown in the Slovak Republic (SR) and can cause significant yield losses and quality reductions. The disease is associated with the occurrence of mycotoxins in kernels. Breeding wheat for resistance to FHB reduces damage and toxin levels in wheat. The resistance in wheat is usually measured by its the spike infection, number of infected kernels, reduction in yield components, and amount of mycotoxin accumulation in kernels. The direct quantification of *Fusarium* in plant material, molecular and immunological methods, were shown to efficient for evaluation of infection in kernels. The winter wheat Slovak cultivars and lines derived from a cross of wheat with related species *Triticum macha* Dekapr. et Menabde, *T. polonicum* L., *T. diccocoides* (Koern. ex Aschers. et. Graeb.) Schweinf. were evaluated for a content of mycotoxin deoxynivalenol (DON) and a kernel infection by *Fusarium* protein equivalent (FPE) and real-time PCR after artificial inoculation with the fungus *Fusarium culmorum* (W. G. Sm.) Sacc. A positive correlation between DON production and *Fusarium* content were confirmed if ELISA and real-time PCR were used to quantify the pathogen. The obtained results indicated that quantification of *Fusarium* in kernels refined the characterisation of genotypes for *F. culmorum* resistance and can be helpful for evaluating wheat genotypes for *Fusarium* infection.

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Development of a Marker Assisted Selection program for the improvement of durum wheat (*Triticum durum* Desf.)

V.M. Menzo[1], A.M. Mastrangelo[1], L. Cattivelli[1,2], R. Papa[1], P. De Vita[1]

[1]Cereal Research Centre (CRA-CER), Agricultural Research Council, S.S 16 km 675, 71122 Foggia (Italy)
[2]CRA-Genomics Research Centre Via S. Protaso, 302, 29017, Fiorenzuola d'Arda (PC) (Italy)

Italy is the main producer of pasta in the world and the genetic improvement of durum wheat represents a strategic activity for the entire agro-industrial sector. DNA markers have an enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS). This paper describes the work carried out at the Cereal Research Centre (CRA-CER) for the development of a MAS breeding program dedicated to the pyramiding of genes for low lipoxygenase (LOX) activity (*Lpx-B1.1*), high protein content (GPC; *Gpc-B1*), high yellow pigment content (YPC; *Psy-A1*) and disease resistances. The following R genes were considered: leaf, stripe and stem rust (*Lr14c*, *Yr36* and *Sr13* or *Sr26*, respectively), powdery mildew (*Pm36*) and soil borne cereal mosaic virus (SBCMV; *QSbm.ubo-2BS*). A set of durum wheat varieties and introgression lines carrying the desirable genes were chosen as donor lines, while the recipient line was the Italian durum cultivar PR22D89, characterized by a high gluten quality and good yield. The crosses were performed separately for each donor line with PR22D89, than the introgressed genes were first fixed in a homozygous after the screening of the F_2 populations. Then, the F_2/F_3 plants homozygous for the same genes and meeting the required phenotypic standards were selected for further crosses in order to combine up to 4 genes of interest segregating in the same populations. Presently, several hundreds of genotypes are under evaluation and some $F_{3:5}$ lines are carrying genes at the homozygous state combining four different traits of interest: high GPC, low LOX activity, resistance to stripe rust and powdery mildew; as well as high GPC, low LOX activity, resistance to stripe and leaf rust. These lines exhibited a good increase in GPC with a very limited negative impact on grain kernel weight.

Marker-assisted pyramiding of four grain quality traits and leaf rust resistance in Indian bread wheat cv. PBW343

S. Tyagi[1], R.R. Mir[1, 2], S. Kumar[1], P. Chhuneja[3], H.S. Balyan[1], P.K. Gupta[1]

[1]Molecular Biology Laboratory, Department of Genetics and Plant Breeding, Ch. Charan Singh University, Meerut - 250 004, UP, India
[2]Centre of Excellence in Genomics (CEG), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru - 502 324, Greater Hyderabad, India
[3]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana - 141 004, Punjab, India

During the last 15 years, our laboratory has been engaged in development and use of DNA-based markers for marker-assisted selection for some quality traits in wheat. These quality traits included pre-harvest sprouting tolerance (PHST), grain protein content (GPC) and grain weight (GW). More recently markers associated with these and other quality traits were used for marker assisted selection (MAS) for improvement of a number of high-yielding wheat cultivars. One such cultivar is the widely grown high yielding cultivar PBW343. However, this cultivar has moderate grain quality and has recently become susceptible to leaf and stripe rusts. We attempted pyramiding of QTLs/genes for four grain quality traits (PHST, GPC, GW and gluten strength) and one leaf rust resistance gene in the background of this cultivar. Four genetic stocks were initially produced and intercrossed to produce the following F_1 hybrids: PBW343 (*Lr24+Gpc-B1*)/PBW343 (*QPhs.ccsu-3A.1*) and PBW343 (*Lr24+QGw.ccsu-1A.3*)/PBW343 (*Glu-Ax-Ay*). These F_1 hybrids were again intercrossed to obtain a double-cross hybrid ($DCHF_1$) population, which was subjected to foreground selection for all the QTLs/genes (except the *Glu-Ax-Ay*). Four $DCHF_1$ plants that were heterozygous for all the four QTLs/genes (*Lr24*, *Gpc-B1, QPhs.ccsu-3A.1* and *QGw.ccsu-1A.*3) were selected and used to obtain $DCHF_2$ progenies, which were again subjected to foreground selection. Seed was collected from as many as 16 $DCHF_2$ plants that were found to be homozygous/heterozygous for each of the above four QTLs/genes. Using half seed analysis approach, $DCHF_3$ seeds were analysed for *Glu-Ax-Ay* on SDS-PAGE gels and a total of 19 positive seeds containing *Glu-Ax-Ay* (either in heterozygous or in homozygous condition) were identified; plants were raised from these 19 seeds for foreground selection leading to the identification of five plants each with five QTLs/genes (with homozygosity for PHST and GW). The $DCHF_4$ progenies derived from the above five $DCHF_3$ plants are being currently grown and the foreground selection will be carried out to identify $DCHF_4$ progenies that are homozygous (on the basis of lack of segregation) for all the five QTLs/genes. This should lead to successful pyramiding of five QTLs/genes for grain quality and leaf rust resistance in the genetic background of cv. PBW343.

Implementation of QTL mapping outputs in South African wheat breeding programs

G. Agenbag[1], Z. Pretorius[1], L.Boyd[2], C. Bender[1], R. Prins[1, 3]

[1]Department of Plant Sciences University of the Free State Bloemfontein 9300, South Africa; [2]Department of Disease and Stress Biology John Innes Centre Norwich NR4 7UH, United Kingdom
[3]CenGen (Pty) Ltd Worcester 6850, South Africa

QTL mapping is a powerful tool to dissect the genetic components responsible for disease resistance. The wheat cultivars Kariega and Cappelle-Desprez have complete adult plant resistance to stripe rust. The objective of this study was to unravel their resistance using a QTL mapping approach. Various marker techniques were used to identify QTL in a Kariega X Avocet S doubled haploid mapping population. In addition to the *Lr34*/*Yr18* gene, two QTL, *QYr.sgi-4A* and *QYr.sgi-2B*, were identified which contribute significantly to stripe rust resistance displayed by Kariega. The genetic map resolution of the QTL intervals was improved using a large F_2 mapping population. Stripe rust resistance from Cappelle-Desprez was studied in a Palmiet X Yr16DH70 recombinant inbred line mapping population. Yr16DH70 is a Cappelle-Desprez derivative, which is a more suitable parent in a spring wheat background. A major QTL (*QYr.ufs-2A*) was identified on chromosome 2A and minor QTL on chromosomes 2D (*QYr.ufs-2D*/*Yr16*), 5B (*QYr.ufs-5B*) and 6D (*QYr.ufs-6D).* Markers were identified to track both the Kariega and Cappelle-Desprez QTL in marker-assisted breeding and QTL pyramiding approaches. The South African wheat industry through the Winter Cereal Trust has committed themselves in 2010 to assist wheat breeders in implementing high-throughput marker-assisted selection on a significant scale via the CenGen Molecular Service Laboratory. The markers identified in this study are amongst the many that have already been used to screen the first breeding material received in this new endeavour.

Optimizing the baking quality in common wheat: The case of Cameroon

M.N. Tayor

Thesis submitted in partial fulfillment of the requirement for the
Degree of Master of Science in crop sciences
The Norwegian university of life sciences

In the past, bread was the major wheat product in Cameroon but today, the introduction of non- bread wheat products have increased the wheat demand. With the present increase in wheat demand in Cameroon, there is the need to seek means of promoting domestic wheat production. The overall objective of this work was to investigate the wheat situation in Cameroon and seek means to contribute to increased domestic production of quality bread wheat in Cameroon. In attempt to build competence in wheat quality and improvement, a study of the influence of millers on importers and the influence of bakers on millers on quality requirements was carried out. The study was conducted through a survey among the flour milling companies and the major bakeries. It was found that all the wheat for flour production in Cameroon is imported from different regions of the world. Most of the wheat imported has protein content less than 12 %. It was also found that the milling companies have no influence on imported wheat quality neither do the bakeries have any influence on the millers regarding quality requirements. As the prices of wheat continue to rise, it is most likely that low quality wheat will be exported to Cameroon as the milling companies have no influence on the importers. The study also review increase in non-bread wheat products. In order to gain more knowledge on baking quality of common bread wheat, an analysis of quality traits in a set of wheat varieties selected from diverse origin was grown at two locations in Norway: Vollebekk-UMB and Staur-Graminor. The second part involved laboratory analysis of some wheat baking qualities. The above results highlighted quality requirement for bread wheat that can be grown in Cameroon. In order to secure quality wheat product in Cameroon for the future, there is the need to start domestic wheat production. Recommendations were made on how domestic wheat production in Cameroon could be achieved.

Development of gene specific SNP assays open a new perspective on marker assisted selection in wheat

S. Dreisigacker[1]*, L. Patino Zuniga[1], J. Ceja Navarro[2]

[1]International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico DF, Mexico
[2]Lawrence Berkeley National Laboratory, 1 Cyclotron Rd, Berkeley, California 94720, United States
*E-mail: s.dreisigacker@cgiar.org

To face the demands for increasing global food production, plant breeding is adopting various approaches to develop improved cultivars and increase crop yields. Molecular marker technology offers a wide range of approaches improving selection strategies in breeding. Marker assisted selection (MAS), marker assisted backcrossing and genomic selection have shown to provide valuable assistance to increase selection efficiency through indirect selection for agronomically valuable traits and are therefore increasingly adapted in breeding programs. The discovery of a large number of single nucleotide polymorphisms (SNPs) in crops has accelerated the development of genotyping technology with increased throughput and low cost. However, most platforms are qualified for the screening of large numbers of markers and genotypes simultaneously while in most wheat breeding programs MAS is currently applied using a limited number of markers across large segregating populations during narrow time intervals. For successful incorporation in wheat breeding programs, flexible and multi-parallel genotyping detection methods would be profitable. We have used available and novel sequence information to develop SNP and Indel detection primers using the KASPar technology (KBioscience) linked to genes that are extensively applied in MAS programs in wheat at CIMMYT and worldwide. SNP assays of known polymorphism for *Lr34*, *VPM*, *Fhb1*, *Glu-D1*, *GPC-B1*, *Rht-B1*, *Rht-D1* are presented. SNP assays were validated on a set of wheat lines to assess their suitability for high-throughput MAS. The SNP assays have found to be been robust, reliability and significantly reduce the time and cost which may accelerate the transfer of markers to practical plant breeding.

Posters

Session 4

Exploring and exploiting Triticeae Genetic Resources

Genetic and phenotypic variation in a subset of durum wheat cultivars suitable for association mapping

P. Roncallo[3]*, V. Beaufort[1, 3]*, E. Camargo-Acosta[2, 3]*, I. Garbus[2, 3], V. Echenique[1, 3] * Equal contribution to this work

[1]Departamento de Agronomía. Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina
[2]Departamento de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina
[3]CERZOS (CCTBB, CONICET), Camino La Carrindanga, Km 7. B8000FWB – Bahía Blanca, Buenos Aires, Argentina

We report here the evaluation of a subset of 47 durum wheat [*T. turgidum* subsp. *durum* (2n = 4x = 28)] cultivars from diverse origins (Argentina [28], Italy [13] and CIMMyT [6]) from a complete population of 154 materials suitable for association mapping studies. We analyzed these materials for yield, quality and morpho-physiological traits such as grain yield, thousand kernel weight, kernel width, kernel length, test weight, grain protein content, SDS test, flour color (L*a*b*), yellow pigment content, plant height, heading date and flowering date. Durum wheat cultivars were tested in three environments from Argentina (Barrow 2006, Cabildo 2007, Barrow 2007), using a randomized complete block design with three replications, in 3 m^2 plots. Correlation analyses among traits and ANOVA for all the traits were performed. Genetic diversity and relationships among these materials were assessed using five primer combinations of amplified fragment length polymorphisms (AFLP). In addition, allelic variation at the phytoene synthase 1 gene (*Psy-B1*), involved in the biosynthesis of carotenoids pigments, was explored. We detected genotype x environment interaction in all phenotypic traits, with a strong genotype effect on SDS test and yellow pigment content. Thousand kernel weight showed a significant positive correlation with grain yield (r=0.53) and negative correlation with flowering date (r=-0.59), grain protein content (r=-0.55) and flour b*(r=-0.49). Three thousand and thirty five (335) loci were screened with AFLP yielding a total of 160 (47.8%) polymorphic bands. An average of 32 polymorphic loci (ranging from 17 to 55) per primer combination was obtained. Unique bands by origin (Argentina [9], Italy [2]) were detected and significant differences among variety groups from Italy and Argentina (PhiPT=0.226, $p<0.01$) and from CIMMyT and Argentina (PhiPT=0121, $p<0.05$) were found by AMOVA. UPGMA cluster analysis, based on the simple matching coefficient, showed that CIMMyT materials clustered together, whereas Argentinean and Italian cultivars had different degrees of genetic relationships. At least two alleles at the *Psy-B1* locus (*Psy-B1a* and *Psy-B1b*) were found in a preliminary analysis.

The genetic diversity of the *DRF1* gene in durum wheat and its ancestors

D. Di Bianco[1, 2], C. Cantale[1], K. Thiyagarajan[1, 2], K. Ammar[3], P. Galeffi[1]

[1]ENEA, UTAGRI-GEN, Rome, Italy
[2]Agrobiodiversity International PHD SCHOOL, S. Anna Superior School, Pisa, Italy
[3]CIMMYT, El Batan-Ciudad Obregón, México

The *DRF1* (dehydration responsive factor 1) gene belongs to DREB-family and is involved in the abiotic stress response. The same alternative splicing mechanism characterizing the *DRF1* gene expression in wheat was also observed in its wild relatives. The genetic diversity of DRF1 gene has been investigated in durum wheat, *Triticum urartu*, *Aegilops speltoides* and in other *Aegilops*. The *DRF1* gene was characterized in around 100 durum accessions, more than 200 wild ancestor accessions and in a RIL durum wheat population consisting of 177 inbred lines. SNP, SSR and insertions/deletions were identified in view of gaining more knowledge about the intra- and inter-specific variability of the *DRF1* gene in its different forms and homeologous copies. A PCR selective test based on the gene sequence was set up and used for clustering the RIL population.

Comparison of the grain yellow pigment content (GYPC) between Chilean durum wheat (*Triticum turgidum* L.ssp. *durum*) and international wheat cultivars

A. Schulthess, A.R. Schwember

Department of Plant Science, Faculty of Agronomy and Forest Engineering, Pontifical Catholic University of Chile (PUC), Santiago of Chile

The bright yellow color of semolina obtained from grains of durum wheat is considered a quality trait in different parts of the world. The Chilean cultivars have insufficient levels of grain yellowness that does not satisfy the international standards of this trait. Chile possesses some comparative advantages to produce high quality grains of durum wheat that are currently not being fully exploited. To date, there is no reported comparison available of national genotypes with variable grain yellowness in a given environment. In this study, we present a single environment micro-assay in the 2010-2011 season, which concluded that there is a strong genotypic component regulating grain yellow pigment content (GYPC). In addition, the present GYPC of the main Chilean commercial cultivars of durum wheat is unsatisfactory to meet the international color standards associated with high quality, reflecting the need for breeding on this particular trait within the Chilean breeding programs.

Effects of *Ppd-A1* and *Ppd-B1* alleles on the phenology of durum wheat grown at contrasting latitudes

D. Villegas[1], C. Royo[1], K. Ammar[2], L.F. García del Moral[3], M. M. Cátedra[4], S. Dreisigacker[2]

[1]Institut de Recerca i Tecnologia Agroalimentàries (IRTA). Av. Alcalde Rovira Roure 191. 25198. Lleida. Spain
[2]CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo), Km 45 carretera Mex-Veracruz, el Batan, Texcoco, 56130 Mexico
[3]Departamento de Fisiología Vegetal. Universidad de Granada. Av. Fuentenueva s/n. 18071 Granada. Spain
[4]Instituto de Investigación y Formación Agraria y Pesquera (IFAPA). Rancho de la Merced. Ctra. de Trebujena km 3.2. 11471 Jerez de la Frontera. Spain

Photoperiod sensitivity and vernalization response are main genetic factors regulating time to heading. This research aimed to study the effect of the latitude on the expression of the major genes regulating the photoperiod sensitivity in durum wheat. Field experiments, involving 41inbred lines with different allele combinations for the *Ppd-A1* and *Ppd-B1* loci, but equally dominant for *Vrn-A1*, were conducted during two years at four contrasting latitudes (41º38'N Spain-north, 37º0'N Spain-south, 27º21'N Mexico-north, and 19º31'N Mexico-south). The ANOVA revealed that the effects of the genotype, latitude and the genotype x latitude interaction accounted for 40.2%, 39.5% and 12.0% respectively, of total variance for thermal-time from sowing to heading. In autumn-planted experiments thermal time until heading significantly increased when moving from northern to southern sites. The shortest thermal-time until heading was recorded in experiments planted during the spring in southern Mexico, which had the longest daylength in the pre-heading period. The clustering of the genotypes according to the alleles present in the *Ppd-A1* and *Ppd-B1* loci explained 50% of the variability attributed to genotype in thermal-time until heading. The largest variability for cycle length until heading was recorded in genotypes carrying the *Ppd-A1* allele conferring photoperiod sensitivity. The earliest genotypes had the *Ppd-A1* alleles conferring photoperiod insensitivity, while the combination of *Ppd-A1* sensitive with *Ppd-B1* insensitive produced the latest genotypes. The alleles present in the *Ppd* loci interacted with the latitude effect in the expression of the cycle duration until heading.

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Identification and Mapping of a New Leaf Rust Resistance Gene derived from *triticum turgidum* var. *dicoccum*

F. Desiderio[1], A.M. Mastrangelo[2], D. Rubiales[3], M. Pasquini[4], R. Simeone[5], A. Blanco[5], L. Cattivelli[1], G. Valè[1]

[1]CRA–Genomics Research Centre, Via S. Protaso, 302, 29017 Fiorenzuola d'Arda (PC) – Italy
[2]CRA-Cereal Research Centre, Foggia, Italy
[3]Instituto de Agricoltura Sostenibile(CSIC), Cordoba, Spain
[4]CRA-Experimental Institute for Cereal Research, Roma, Italy
[5]Department of Environmental and Agro-Forestry Biology and Chemistry , Bari, Italy

Leaf rust, caused by the fungus *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*) is one of the most important diseases for wheat, causing significant yield losses annualy in many wheat growing regions of the world. The utilization of resistance genes is the most viable and economical strategy to minimize the yield losses, nevertheless, the source of resistance gene is rather limited in durum wheat genetic background, a situation that requires the search of new resistance genes in related wheats. In order to investigate the genetic basis of new leaf rust resistances, we are currently developing a genetic linkage map on a RILs population (122 F9 lines) derived from a cross between the susceptible durum wheat cultivar Latino and the resistant accession MG5323 of *T. turgidum* var. *dicoccum*. The phenotypic characterization (Infection Type and Relative Disease Severity) of the RIL population by means of artificial inoculations with two *P. triticina* isolates (Jerez 05 and 16081-1) was performed in greenhouse experiments carried out in Italy and Spain. More than 400 microsatellite markers with known position and uniformly distributed on the whole genome were tested on the parents of the segregating population, and 79% of them were found polymorphic. A genetic linkage map for QTL analysis was therefore developed using about 300 SSR markers distributed within the 14 linkage groups and spanned greater than 2000 cM. The QTL analysis carried out using the disease response data (IT and RDS scoring) on all the RILs has allowed to uncover one major QTL localized in a genomic region where no previously identified leaf rust resistance genes (Lr genes) have been positioned. This major QTL was mapped on the short arm of 1B chromosomes and strictly associated SSR markers were identified. This work has therefore resulted in the identification of a new resistance gene to leaf rust in the *durum* wheat genetic background.

The genetic basis of *Fusarium* head blight resistance in CIMMYT bread wheat line SHA3/CBRD

Q. Lu[1], M. Lillemo[1], H. Skinnes[1], H. Xinyao[1], J. Shi[2], F. Ji[2], A. Bjørnstad[1]

[1]Dept. of Plant and Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway
[2]Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

Fusarium head blight (FHB) is a destructive wheat disease of global importance. Resistance breeding efforts around the globe depend heavily on Sumai-3 and its derivatives with the *Fhb1* gene. Hence, there is a need to broaden the genetic base of resistance. Shanghai-3/Catbird (SHA3/CBRD) is a promising source of resistance that is not based on Sumai-3. A recombinant inbred line (RIL) population from the cross of SHA3/CBRD with the German spring wheat cv. Naxos was evaluated for FHB resistance and related traits in field trials in Norway and China. Included were two trials with point inoculation, two trials with spray inoculation and one inoculated with infected oat kernels spread on the ground. Simple trait correlations revealed no association between head blight severities after point inoculation and spray inoculation. Anther extrusion showed a strong correlation with head blight severity after spray inoculation, while the association with *Fusarium*-damaged kernels (FDK) and DON was only moderate and not always significant. The population segregated for the *Rht-B1b* dwarfing allele, which was associated with increased susceptibility after spray inoculation, but not after point inoculation. Overall, this locus explained about 7% of the phenotypic variance in head blight severity after spray inoculation. Several minor QTL for plant height were detected and the alleles for reduced plant height at these loci were always associated with increased susceptibility after spray inoculation. SHA3/CBRD was found to contribute a major resistance QTL close to the centromere on 2DL affecting head blight severity and DON after both inoculation methods. This QTL was also associated with plant height and anther extrusion, with favourable alleles contributed by SHA3/CBRD. Most other minor QTL with head blight resistance from SHA3/CBRD were associated with anther extrusion, while QTL with resistance contributed by Naxos were mostly not associated with anther extrusion. While SHA3/CBRD contributed most of the favourable alleles for reduced head blight severity after spray inoculation, Naxos contributed more favourable alleles for reduction in FDK and DON and to FHB resistance after point inoculation. This study has shown that the FHB resistance of SHA3/CBRD and Naxos is a complex trait involving many loci of relatively small effects, but the use of different inoculation methods, assessment of different disease parameters (head blight severity, FDK and DON), and consideration of related traits like anther extrusion and plant height made it possible to dissect the trait into its specific components and gain insight into the possible function of individual QTL. Marker-assisted selection based on the 2DLc QTL from SHA3/CBRD combined with phenotypic selection for anther extrusion is recommended for resistance breeding based on this valuable source of resistance.

Functional characterization of cereal UDP-glucosyltranferases for their ability to inactivate the Fusarium toxin deoxynivalenol

W. Schweiger, P. Kovalsky, F. Berthiller, M. Lemmens, S. Shin, J. Boddu, G.J. Muehlbauer, G. Adam

Department for Agrobiotechnology (IFA), Institute for Biotechnology in Plant Production, Tulln, Austria

The plant pathogenic fungus *Fusarium graminearum* produces the trichothecenes deoxynivalenol (DON) or nivalenol (NIV). The genetic differences between chemotypes predate speciation and both chemotypes coexist in field populations. The protein biosynthesis inhibitors DON and NIV seem to as act as defense suppressors, which can be detoxified to a variable extent into glucosides in different host plants and genotypes by UDP glucosyltransferases (UGT). Diploid grass genomes contain about 150 UGT genes (≈450 in hexaploid wheat!). Microarray data generated from Fusarium challenged barley was used to identify relevant UGT genes in barley. We have tested 7 upregulated UGT genes by heterologous expression in a toxin sensitive yeast strain for their ability to transform a variety of trichothecene toxins into their respective glucosides. Among those barley UGTs tested, one cDNA not only conferred resistance to DON (Schweiger et al, 2010) but also to NIV and fusarenon-X (unpublished). Adding DON to the growth medium of liquid yeast cultures expressing the UGT resulted in the accumulation of DON-glucoside in the cells and in the medium. To test whether homologous genes in related species display similar substrate specificity we have identified 136 UGT genes in the model grass Brachypodium distachyon and constructed a phylogenetic tree, which was used to relate the barley and other candidate UGT genes. From this comparison emerged a cluster of six Brachypodium genes, which have the highest amino acid sequence similarity to the trichothecene detoxifying barley UGT. Cloning and testing showed, that two of the candidate Brachypodium UGTs conferred resistance to DON and one of these two genes was also able to metabolize an acetylated DON derivative (15ADON) and NIV. These UGTs should be valuable for Fusarium control, since they cannot be easily overcome by a shift in chemotype composition in the pathogen.

Wheat blast, a menace for tropical agriculture

G. Torres[1], L. Consoli[1], S. Bonow[2], A. Goulart[3], A. Roese[3], C. Seixas[4], A. Dianese[5], J. Pereira[1], A.L. Bonato[1], J. Maciel[1]

[1]Embrapa Wheat, Cx. Postal 451, 99001-970, Passo Fundo-RS, Brazil
[2]Embrapa Temperate Agriculture, Cx. Postal 403, 96010-971, Pelotas-RS, Brazil
[3]Embrapa Western Region Agriculture, Cx. Postal 661, 79804-970, Dourados-MS, Brazil
[4]Embrapa Soybean, Cx. Postal 231, 86001-970, Londrina-PR, Brazil
[5]Embrapa Cerrados, Cx. Postal 08223, 73310-970, Planaltina-DF, Brazil

Wheat blast was recorded for the first time in 1985 in Brazil. To date, it is spread to Bolivia, Paraguay and Argentina. *Magnaporthe oryzae* can infect wheat seedlings and adult plants but the most severe symptoms are observed in spikes which become bleached. The fungus prevents grain-filling or destroys the grain-bearing structures. Few wheat cultivars have been identified as resistant, depending on natural innoculum pressure. Since 2009, Embrapa coordinates a national program turned to the identification of resistance sources for blast disease and elucidation of mechanisms of plant-pathogen interaction exploring genetic resources under controlled and field conditions. Phenotyping activities under controlled conditions have been conducted with 80 wheat lines and cultivars originated from different countries in the world. Since blast is highly dependent on temperature and humidity, methodological procedures of spikes inoculation were standardized. After the identification of resistant wheat genotypes to blast disease submitted to high inoculation pressure, molecular approaches have been conducted to characterize differential expression profiles at transcriptional and translational levels. In 2010, the first nurseries of wheat blast were conducted in three locals in Brazil: Dourados-MS, Londrina-PR and Planaltina-DF, where high temperature and high humidity conditions are favorable to the occurrence of blast disease. Two-hundred wheat genotypes could be evaluated under natural infection conditions. Although 2010 was not a year with high blast incidence levels in wheat fields, the hot spots experimental design worked as well as it was possible to identify resistance differences among the genotypes evaluated. The same group of cultivars showed different disease incidence depending on the location: we speculate the occurrence of divergent pathogen population in three Brazilian regions. Another group of cultivars revealed the best performance in different sites: probably these genotypes present resistance to a broad range of *M. oryzae* isolates. First crosses among wheat resistant genotypes were made to obtain wheat lines with better resistance levels and to generate mapping populations to study the genetic basis of disease resistance. The wheat blast hot spots are being conducted in 2011 and are to be established in other sites of evaluation in Brazil in the next years.

Identification of wheat-barley addition lines using EST markers mapped in barley

T. Koba[1], K. Mishina[1, 2], T. Kawaguchi[1], S. Kikuchi[1], A. Ijaz[1, 3], K. Nagataki[1], H. Sassa[1], K. Sato[4]

[1]Graduate School of Horticulture, Chiba University, Matsudo, Chiba 271-8510, Japan,
[2]Laboratory of Plant Genome Science, Kihara Institute for Biological Research, Yokohama City University, 641-12 Maioka, Totsuka, Yokohama 244-0813, Japan,
[3]Science Superior College, Peshawar, Pakistan,
[4] Research Institute for Bioresources, Okayama University, Kurashiki 710-0046, Japan

Eighteen disomic barley chromosome addition lines of common wheat were tested for identification of added chromosomes or fragments introgressed into wheat using EST SNP markers of barley, which were mapped on the barley chromosomes. The addition lines were produced from the hybrids between Japanese wheat cultivars, Shinchunaga and Norin12, as female parents, and Nyugoruden (New Golden) and Haruna Nijo as pollinators. Ninety-six EST primers corresponding to each barley chromosome were tested. All of the primers tested amplified the bands specific to barley chromosomes. Product sizes of some primers were common to wheat and barley, which were excluded from the test. As the result, three lines were detected as the whole chromosome addition lines of chromosome 2H, one line of 3H, two lines of 4H, two lines of 5H, one line of 6H and no lines of 7H. Short arm of 3H and short arm of 5H were detected in three and one lines, respectively, which were considered to be translocated with wheat chromosomes. These results were also confirmed with GISH method. The result showed the usefulness of the EST SNP markers for detection of the barley chromosomes in wheat-barley chromosome addition lines.

Posters

***Session** 5*
Bioinformatics and Computational Biology

TriAnnot: A High Performance Pipeline for the Automated Structural and Functional Annotation of Plant Genomes

P. Leroy[1], N. Guilhot[1], S. Theil[1], A. Bernard[1], M. Alaux[2], S. Reboux[2], F. Choulet[1], H. Sakai[3], T. Tanaka[3], T. Itoh[3], H. Quesneville[2], C. Feuillet[1]

[1]INRA-University Blaise Pascal Joint Research Unit 1095 Genetics, Diversity and Ecophysiology of Cereals, Clermont-Ferrand 63100, France
[2]INRA Research Unit 1164 Genomics-Informatics, Versailles 78026, France
[3]National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

To achieve a systematic and comprehensive annotation of the bread wheat (*Triticum aestivum* L.) genome sequence (17 Gbp, 2n=6x=42, AABBDD), we are developing under the umbrella of the IWGSC (http://www.wheatgenome.org), an automated annotation pipeline called TriAnnot. The aim is to provide the international scientific community with an online user-friendly interface for BAC annotation and facilitate large scale analysis on whole genome or whole chromosome sequences, such the 1Gb sequence of chromosome 3B currently under analysis in the framework of the ANR/FranceAgriMer 3BSEQ project. To cope with the bioinformatic challenge of annotating more than 1200 BAC contigs, a version adapted to parallel computing (V3.0) has been developed. The pipeline has potentially access to 705 cores which will enable the structural and functional automatic annotation of the ~1Gb of the chromosome 3B reference sequence in less than 5 days. The modular architecture of TriAnnot allows for the identification and annotation of repeats and Transposable Elements (TEs), protein-coding genes, RNA-coding genes as well as other biological features. Annotations can be retrieved through EMBL (EMBL output files have been formatted to be used easily with GenomeView (http://genomeview.org/), ARTEMIS (http://www.sanger.ac.uk/resources/software/artemis/)) and GFF files. They are also inserted automatically into a relational database that is connected to an online GBrowse. The manually curated annotations will be integrated into the URGI Information System (http://urgi.versailles.inra.fr/gnpis/) that provides links beween genetic and physical maps, QTL and markers information as well as phenotypic data within a single platform. The performance of TriAnnot was evaluated based on a set of 148 genes manually curated (Choulet et al. 2010) and compared with other pipelines (MIPS, RiceGAAS and FPGP). The sensitivity (*Sn*) and specificity (*Sp*) values demonstrated that the TriAnnot pipeline perfectly predicts and annotates more than 50% of the genes and has a higher fitness than the other pipelines. TriAnnot can be adapted easily to the annotation of other plant genomes with minor modifications. A full description and access to the TriAnnot pipeline is available at http://www.clermont.inra.fr/triannot.

Haplotype Analysis of the *Rdg2a* Locus in Different Barley Varieties

C. Biselli[2], A. Martini[1], S. Urso[1], L. Cattivelli[1], G. Valè[1, 2]

[1]Genomic Research Center, CRA-GPG, Via S. Protaso 302, I-29017 Fiorenzuola d'Arda (PC), Italy
[2]Unità di ricerca per la risicoltura, CRA-RIS, Strada Statale 11 per Torino km 2,5, 13100, Vercelli, Italy

Leaf stripe disease on barley is caused by the seed-transmitted hemi-biotrophic fungus *Pyrenophora graminea*. Race-specific resistance to leaf stripe is controlled by two known *Rdg* (*R*esistance to *Drechslera graminea*) genes: the *H. spontaneum*-derived *Rdg1a*, mapped to chromosome 2HL and *Rdg2a*, identified in *H. vulgare*, mapped on chromosome 7HS and cloned in the resistant cultivar (cv.) Thibaut. The *Rdg2a* locus contains a gene cluster of three sequence-related Coiled-Coil, Nucleotide-Binding site, and Leucine-Rich Repeat (CC-NB-LRR) encoding genes. However, only one gene conferred resistance to isolate *Dg2*, against which *Rdg2a* is effective, when the susceptible cv. Golden Promise was transformed with the *Rdg2a*-candidates. The high level of sequence similarity between the three genes most likely contributed to significant rearrangements during evolution. Un-equal crossing-overs probably occurred and resulted in sequence exchange between paralogs and in the generation of recombinant genes, as well as in expansion/contraction of gene copy number. To examine haplotype variation at the *Rdg2a* locus, the sequencing of the allelic *Mrdg2a* (Morex *rdg2a*) locus of the leaf stripe susceptible barley cv. Morex was carried out and revealed large rearrangements including two deletions that generated an *Rdg2a*-homolog gene. This gene most likely derived from an un-equal crossing-over between the *Rdg2a* ancestor and its paralog *Nbs2-Rdg2a*. PCR analyses performed with informative markers at five loci within the *Rdg2a* locus identified four different haplotypes. The Thibaut haplotype was observed to be largely conserved in *Dg2*-resistant barley cultivars. The re-sequencing of the *Rdg2a* gene in barley genotypes showing the same Thibaut haplotype or the same resistant phenotype revealed high sequence similarity to Thibaut *Rdg2a*, demonstrating the widespread conservation of the gene. Nonetheless, some sequence variations were identified in at least two barley genotypes that were verified for possible differences, with respect to *Rdg2a*, in the range of resistance specificities towards different leaf stripe isolates.

List of Participants

Abdissa, Dawit Fekru
Human Develpoment Assocation
Agri Dep. Hade
Saris
19090, Addis Ababa
ETHIOPIA
lidudawit6@gmail.com

Agenbag, Gloudi
University of the Free State
Department of Plant Sciences
78 Fairbairn Street
6850, Worcester
SOUTH AFRICA
gloudi.agenbag@bbsrc.ac.uk

Akhunov, Eduard
Kansas State University
Plant Pathology
Throckmorton Plant Sciences Center
Manhattan
UNITED STATES
eakhunov@ksu.edu

Alaux, Michael
INRA
Centre INRA de Versailles, URGI Bat 18, Route de Saint-Cyr
78026, Versailles
France
michael.alaux@versailles.inra.fr

Ammar, Karim
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
k.ammar@cgiar.org

Bains, Navtej
Punjab Agricultural University
Plant Breeding and Genetics
Ferozepur Road
141004, Ludhiana
INDIA
nsbains@rediffmail.com

Barker, Chris
Genome Prairie
101-111 Research Dr
S7N 3R2, Saskatoon
CANADA
cbarker@genomeprairie.ca

Bhavani, Sridhar
CIMMYT, Kenya
P.O. Box 1041 Village Market 00621, Nairobi, Kenya
Nairobi
KENYA
s.bhavani@cgiar.org

Bjornstad, Aasmund
Norwegian University of Life Sciences
Dept. of Plant and Environmental Sciences
P.O. Box 5003
1432, Ås
NORWAY
asmund.bjornstad@umb.no

Bonnett, David
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
d.bonnett@cgiar.org

Braun, Hans - Joachim
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
h.j.braun@cgiar.org

Brennan, Paul
CropGen International
PO Box 9055
2480, Rock Valley
AUSTRALIA
paul.brennan@bigpond.com

Brown, Guedira Gina
USDA-ARS Plant Science Research
4114 Williams Hall
27695, Raleigh, NC
UNITED STATES
gina_brown-guedira@ncsu.edu

Buerstmayr, Hermann
BOKU - University of Natural Resources and Life Sciences, Vienna
Department for Agrobiotechnology Tulln, Institute for Biotechnology in Plant Production
Konrad Lorenz Str. 20
3430, Tulln
AUSTRIA
hermann.buerstmayr@boku.ac.at

Caccamo, Mario
The Genome Analysis Centre
Bioinformatics
Norwich Research Park
NR47UH, Norwich
UNITED KINGDOM
helen.tunney@tgac.ac.uk

Cantale, Cristina
ENEA Italian National agency for new technologies, Energy and sustainable economic development
UTAGRI-GEN
via Anguillarese, 301,
123, Rome
ITALY
cantale@enea.it

Cativelli, Mariana
Instituto Nacional de Tecnología Agropecuaria (INTA)
Instituto de Recursos Biológicos (IRB)
De los Reseros y Nicolas Repetto
without number
1686, Hurlingham
ARGENTINA
mcativelli@cnia.inta.gov.ar

Cavanagh, Colin
CSIRO
Plant Industry
Clunies Ross St
2601, Canberra
AUSTRALIA
colin.cavanagh@csiro.au

Clavijo, Bernardo
Instituto Nacional de Tecnología Agropecuaria
Nicolas Repetto y Los Reseros
Hurlingham
ARGENTINA
bjclavijo@gmail.com

Consoli, Luciano
Embrapa Wheat
Plant Breeding and Biotechnology
Rod. BR 285, km 294
C.Postal 451
99001970, Passo Fundo
BRAZIL
consoli@cnpt.embrapa.br

Crossa, Jose Luis
CIMMYT Int.
Apdo Postal 6-641
6600, Mexico
MEXICO
j.crossa@cgiar.org

Diéguez, María José
INTA
Instituto de Genética "Ewald A. Favret"
CC25
1712, Castelar
ARGENTINA
mdieguez@cnia.inta.gov.ar

Doležel, Jaroslav
Centre of the Region Haná for Biotechnological and Agricultural Research
Institute of Experimental Botany
Sokolovska 6
77200, Olomouc
CZECH REPUBLIC
dolezel@ueb.cas.cz

Dreisigacker, Susanne
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
sdreisigacker@cgiar.org

Dubcovsky, Jorge
Dept. of Plant Sciences
University of California
One Shields Av.
CA 95616-8515, Davis
UNITED STATES
jdubcovsky@ucdavis.edu

Dubreuil, Pierre
BIOGEMMA
Department of Crop Genetics
La Garenne - Route d'Ennnezat
63720 Chappes,
FRANCE
pierre.dubreuil@biogemma.com

Dugo, Tesfaye Letta
Bologna University
Department of Agroenvironmental Sciences and Technology
viale Fanin 44
40127, Bologna
ITALY
tesfayeletta@gmail.com

Duveiller, Etienne
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
e.duveiller@cgiar.org

Dvorak, Jan
University of California
Plant Sciences
1 Shields Avenue
95616, Davis
UNITED STATES
jdvorak@ucdavis.edu

Echenique, Viviana
CONICET
CERZOS
San Andres 800
8000, Bahía Blanca
ARGENTINA
echeniq@criba.edu.ar

Ellis, Marc
CIMMYT Int.
Apdo Postal 6-641
6600, Mexico
MEXICO
mhe.mex@gmail.com

Eversole, Kellye
Eversole Associates/IWGSC
5207 Wyoming Road
20816, Bethesda, MD
UNITED STATES
eversole@eversoleassociates.com

Feuillet, Catherine
INRA
Genetics Diversity and Ecophysiology of Cereals
234 Avenue du Brezet
63100, Clermont-Ferrand
FRANCE
catherine.feuillet@clermont.inra.fr

Fleury, Delphine
Australian Centre for Plant Functional Genomics
University of Adelaide
PMB1
SA5064, Glen Osmond
AUSTRALIA
delphine.fleury@acpfg.com.au

Ganal, Martin
TraitGenetics GmbH
Am Schwabeplan 1b
6466, Stadt Seeland OT Gatersleben
GERMANY
ganal@traitgenetics.de

Garbus, Ingrid
CERZOS/CONICET
Camino "La Carrindanga" km 7
Bahia Blanca
ARGENTINA
igarbus@criba.edu.ar

Gerjets, Tanja
UMR INRA-UBP 1095 Genetics, Diversity and Ecophysiology of Cereals (GDEC)
234 Avenue du Brezet
63100, Clermont-Ferrand
FRANCE
tanja.gerjets@clermont.inra.fr

Gharbi, Mohamed Salah
National Institute of Agriculture Research, Tunisia
Field Crop Lab
Rue Hedi Karray
2049 Ariana,
TUNISIA
gharbi.medsalah@iresa.agrinet.tn

Giradeau, Pascal
SECOBRA Researches
Centre de Bois Henry
78580, Maule
FRANCE
Pascal.giradeau@secobra.com

Gosman, Nicolas
National Institute of Agricultural Botany (NIAB)
Genetics and Breeding
Huntington Road
CB3 0LE, Cambridge
UNITED KINGDOM
nick.gosman@niab.com

Gregova, Edita
Plant Production Research Center Piestany
Bratislavska cesta 122
921 68, Piestany
SLOVAKIA
gregova@vurv.sk

Grosz, Michael
Monsanto Company
800 N. Lindbergh Blvd
63167, St. Louis
UNITED STATES
michael.d.grosz@monsanto.com

Guerreiro, Laurent
ARVALIS institut du vegetal R&D
3 rue joseph et marie Hackin
75116, Paris
FRANCE
l.guerreiro@arvalisinstitutduvegetal.fr

Hamza – Ben Youseef, Sonia
Institut National Agronomique of Tunisia
Agronomy and Plant Biotechnology
Charles Nicolle 43
1082 Tunis,
TUNISIA
hamza.sonia@inat.agrinet.tn

Handa, Hirokazu
National Institute of Agrobiological Sciences
2-1-2, Kan-non-dai
305-8602, Tsukuba
JAPAN
hirokazu@affrc.go.jp

Hayden, Matthew
Department of Primary Industries Victoria
Victorian AgriBiosciences Center
1 Park Drive
3083, Bundoora
AUSTRALIA
matthew.hayden@dpi.vic.gov.au

He, Xinyao
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
xinyao.he@umb.no

Hegarty, Joshua
University of California Davis
Plant Sciences
1 shields avenue mail stop 4
48103, Davis
UNITED STATES
jmhegarty@ucdavis.edu

Helguera, Marcelo
INTA EEA Marcos Juarez
Ruta 12 S/N
Marcos Juarez
ARGENTINA
mhelguera@mjuarez.inta.gov.ar

Herzog, Eva
Justus Liebig University Giessen
Institute of Agronomy and
Plant Breeding II
Heinrich-Buff-Ring 26-32
35392, Giessen
GERMANY
eva.herzog@uni-giessen.de

Hirshman, Arik
Agridera Ltd. Wheat
Zeraim farm p.o box 103
70750, Gedera
ISRAEL
arik@agridera.com

Holdgate, Sarah
Ragt Seeds Ltd
Grange Road
Cb10 1ta, Ickleton
UNITED KINGDOM
sholdgate@ragt.fr

Husasin, Tariq
Kanzo Quality Seeds
Research and Development
149-A District Jail road Multan
60000, Multan
PAKISTAN
tariqpbg@hotmail.com

Huluka, Ayele Badebo
Ethiopian Agricultural Research Organization
Debre Zeit Agricultural Research Center
(DZARC), Debre-Zeit, Addis Ababa
ETHIOPIA
ayele88@yahoo.com

Huynh, Bao Lam
Australian Centre for Plant Functional
Genomics
University of South Australia
Hartley Grove
5064, Urrbrae
AUSTRALIA
lam.huynh@unisa.edu.au

Jacobs, Jonny
Bayer BioScience NV
Technologiepark 38
9052, Zwijnaarde
BELGIUM
j.jacobs@bayer.com

Keller, Beat
University of Zürich
Institute of Plant Biology
Zollikerstrasse 107
8008, Zürich
SWITZERLAND
bkeller@botinst.uzh.ch

Khurana, Paramjit
University of Delhi South Campus
Plant Molecular Biology
Dhaula Kuan, Benito Juarez Road
110021, New Delhi
INDIA
param@genomeindia.org

Kianian, Shahryar
North Dakota State University
Plant Sciences
NDSU Dept. 7670, P.O. Box 6050
58108-6050, Fargo, ND
UNITED STATES
s.kianian@ndsu.edu

Koba, Takato
Chiba University
Faculty of Horticulture
648 Matsudo
271-8510, Matsudo
JAPAN
koba@faculty.chiba-u.jp

Komatsuda, Takao
National Institute of Agrobiological Sciences (NIAS)
Plant Genome Research Unit
Kannondai 2-1-2
305 8602, Tsukuba
JAPAN
takao@affrc.go.jp

Korzun, Viktor
KWS LOCHOW GMBH
Cereals Biotechnology
Ferdinand-von-Lochow-Str.5
29303, Bergen
GERMANY
korzun@kws-lochow.de

Krattinger, Simon
CSIRO
Plant Industry
Clunies Ross Street
2601, Canberra
AUSTRALIA
simon.krattinger@csiro.au

Krugman, Tamar
Haifa University
Institute of Evolution
Mt. Carmel
31905, Haifa
ISRAEL
tkrugman@univ.haifa.ac.il

Laroche, Andre
Agriculture and Agri-Food Canada
Lethbridge research Centre
5403 1st Avenue S.
T1K 5G8, Lethbridge
CANADA
andre.laroche@agr.gc.ca

Lawley, Cynthia Taylor
Illumina
25861 Industrial Blvd
94545, Hayward
UNITED STATES
clawley@illumina.com

Lewis, Silvina
Instituto Nacional de Tecnología Agropecuaria (INTA)
Instituto de Recursos Biológicos (IRB)
N. Repetto y Los Reseros s/n
1686, Hurlingham
ARGENTINA
slewis@cnia.inta.gov.ar

Li, Yiwen
Institute of Genetics.and Developmental Biology
No.1 West Beichen Road, Chaoyang District
Beijing
CHINA
ywli@genetics.ac.cn

Li, Yongle
Technische Universität München
Plant Breeding
Emil-Ramann-Str. 4
85354, Freising
GERMANY
hydrogen20002000@yahoo.com.cn

Lidia, Koren
Institute of Genetics and Cytology National Academy of Sciences of Belarus
Akadrmicheskaya 27
Minsk
BELARUS
L.Koren@igc.bas-net.by

Lillemo, Morten
Norwegian University of Life Sciences
Dept. of Plant and Environmental Sciences
P.O. Box 5003
NO-1432, Ås
NORWAY
morten.lillemo@umb.no

Lince, Diane
Illumina, Inc.
Product Marketing
9885 Towne Centre Drive
92121, San Diego
UNITED STATES
dlince@illumina.com

Loladze, Alexander
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
a.loladze@cgiar.org

Lucas, Hélène
INRA
Genetics and Plant Breeding Division
Route de Saint Cyr-RD 10-BÃ¢t 12
78026, Versailles
FRANCE
dgap@versailles.inra.fr

Luo, Ming-Cheng
University of California, Davis
Plant Sciences
One Shields Ave.
95616, Davis
UNITED STATES
mcluo@ucdavis.edu

Maccaferri, Marco
University of Bologna
Dipartimento di Scienze e Tecnologie Agroambientali
Viale Fanin, 44
40127, Bologna
ITALY
marco.maccaferri@unibo.it

Mackay, Ian
NIAB
The John Bingham Laboratory
Huntingdon Road
CB3 0LE, Cambrdige
UNITED KINGDOM
ian.mackay@niab.com

Manés, Yann
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
y.manes@cgiar.org

Marshall, David
The James Hutton Institute
Invergowrie
Dundee
UNITED KINGDOM
David.Marshall@hutton.ac.uk

Mastrangelo, Anna Maria
CRA-Centro di Ricerca per la Cerealicoltura
S.S. 16 km 675
71122, Foggia
ITALY
annamaria.mastrangelo@entecra.it

Matsumoto, Takashi
National Institute of Agrobiological Sciences
Agrogenomics Research Center
2-1-2, Kannondai
305-8602, Tsukuba
JAPAN
mat@nias.affrc.go.jp

Mishina, Kohei
Chiba University
Faculty of Horticulture
648 Matsudo
271-8510, Matsudo
JAPAN
mishina@mvg.biglobe.ne.jp

Mukhopadhyay, Kunal
Birla Institute of Technology
Biotechnology
Mesra
835215, Ranchi
INDIA
kmukhopadhyay@bitmesra.ac.in

Nasuda, Shuhei
Laboratory of Plant Gentics, Graduate School of Agriculture
Kyoto University
Kitashirakawaoiwake-cho
606-8502, Kyoto
JAPAN
nasushu@kais.kyoto-u.ac.jp

Nihorimbere, Gaspard
Burundi Agricultural Institute (ISABU)
Production
Avenue de la cathédrale
Bujumbura
BURUNDI
nihorimbereg@yahoo.fr

Ogbonnaya, Francis
ICARDA
BIGM
Alleppo-Damascus Highway
0, Aleppo
SYRIAN ARAB REPUBLIC
F.Ogbonnaya@cgiar.org

Ogihara, Yasunari
Yokohama City University
Kihara Institute for Biological Research
641-10, Maioka-cho
244-0813, Yokohama
JAPAN
yogihara@yokohama-cu.ac.jp

Olsen, Odd-Arne
Norwegian University of Life Sciences
CIGENE/Department of plant and environmental sciences
Chr. Magnus Falsens vei 1
1432, Ås
NORWAY
odd-arne.olsen@umb.no

Paniego, Norma
Instituto Nacional de Tecnología Agropecuaria
Nicolas Repetto y Los Reseros
Hurlingham
ARGENTINA
npaniego@cnia.inta.gov.ar

Paux, Etienne
INRA
Genetics, Diversity & Ecophysiology of Cereals
234 avenue du Brezet
63100, Clermont-Ferrand
FRANCE
etienne.paux@clermont.inra.fr

Payne, Thomas
CIMMYT Int.
Wheat Germplasm Collection
Apdo Postal 6-641
6600, Mexico
MEXICO
T.Payne@cgiar.org

Poland, Jesse
USDA-ARS
1515 College Ave
66502, Manhattan
UNITED STATES
jesse.poland@ars.usda.gov

Pozniak, Curtis
University of Saskatchewan
Crop Development Centre
51 Campus Drive
S7N 5A8, Saskatoon
CANADA
curtis.pozniak@usask.ca

Quesneville, Hadi
Unité de Recherches en Génomique-Info (UR INRA 1164)
INRA, Centre de recherche de Versailles
RD10, Route de Saint Cyr
78026, Versailles
FRANCE
hadi.quesneville@versailles.inra.fr

Rogers, Jane
The Genome Analysis Centre
Director
Norwich Research Park
NR47UH, Norwich
UNITED KINGDOM
Sasha.stanbridge@tgac.ac.uk

Romero, Jose Rodolfo
Cerzos Conicet, CCT Bahia Blanca, Argentina
Laboratorio de Biotecnologia Vegetal
Carrindanga 7000
8000, Bahia Blanca
ARGENTINA
jromero@criba.edu.ar

Roncallo, Pablo Federico
CERZOS-CONICET/UNS
Dto. de Agronomía
Camino de la carrindanga km 7
8000, Bahia Blanca
ARGENTINA
roncallo@criba.edu.ar

Rosewarne, Garry
CIMMYT China
SAAS
4 Shizishan Rd
610066, Chengdu
CHINA
g.rosewarne@cgiar.org

Royo, Conxita
IRTA
Field Crops
Rovira Roure, 191
E-25198, Lleida
SPAIN
concepcio.royo@irta.es

Saint Pierre, Carolina
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
csaintpierre@cgiar.org

Saintenac, Cyrille
Kansas State University
Plant Pathology
4024 Throckmorton hall
66505, Manhattan
UNITED STATES
saintena@ksu.edu

Sakai, Hiroaki
National Institute of Agrobiological Sciences
2-1-2 Kannondai
305-8602, Tsukuba
JAPAN
hirsakai@affrc.go.jp

Sandve, Simen
Norwegian University of Life Sciences
CIGENE/Department of plant and
environmental sciences
Chr. Magnus Falsens vei 1
1432, Ås
NORWAY
simen.sandve@umb.no

Schnurbusch, Thorsten
Leibniz-Institute of Plant Genetics and Crop
Plant Research (IPK)
Genebank
Corrensstr. 3
6466, Gatersleben
GERMANY
thor@ipk-gatersleben.de

Schweiger, Wolfgang
Institute of Biotechnology in Plant Production
Department for Agrobiotechnology
Konrad Lorenz Strasse 20
3430, Tulln
AUSTRIA
Wolfgang.schweiger@boku.ac.at

Schwember, Andres
Pontificia Universidad Católica de Chile
Ciencias Vegetales
Av. Vicuña Mackenna
4860, Santiago
CHILE
aschwember@uc.cl

Seidensticker, Tina
Leibniz Institute of Plant Genetics and Crop
Plant Research (IPK)
Genebank
Corrensstr. 3
6466, Gatersleben
GERMANY
seidensticker@ipk-gatersleben.de

Shamaya, Nawar
The University of Adelaide
Australian Centre for Plant Fuctional Genomics
Hartley Grove
5064, Urrbrae
AUSTRALIA
nawar.shamaya@acpfg.com.au

Sharp, Peter
University of Sydney
Plant Breeding Institute
107 Cobbitty Road
2570, Cobbitty
AUSTRALIA
peter.sharp@sydney.edu.au

Sharpe, Andrew
National Research Council - Plant
Biotechnology Institute
110 Gymnasium Place
S7N-0W9, Saskatoon
CANADA
fpagdonsolan@genomeprairie.ca

Shatalina, Margarita
Institute of Plant Biology University of Zürich
Zollikerstrasse 107
8008, Zürich
SWITZERLAND
margarita.shatalina@access.uzh.ch

Simkova, Hana
Centre of the Region Hana for Biotechnological and Agricultural Research
Institute of Experimental Botany
Sokolovska 6
CZ-772 00, Olomouc
CZECH REPUBLIC
simkovah@ueb.cas.cz

Singh, Sukhwinder
CIMMYT Int.
Global Wheat Program
Apdo Postal 6-641
6600, Mexico
MEXICO
Suk.singh@cgiar.org

Singh, Kuldeep
Punjab Agricultural University
Biotechnology
Ferozpur Road
141004, Ludhiana
INDIA
kuldeep35@pau.edu

Slikova, Svetlana
Plant Production Research Center Piestany
Bratislavská cesta 122
SK-921 68, Piestany
SLOVAKIA
slikova@vurv.sk

Song, Weining
Northwest A&F University
3 Taicheng RD
712100 Yangling
CHINA
sweining2002@yahoo.com

Sorrells, Mark
Cornell U.
Plant Breeding & Genetics
240 Emerson Hall
14853, Ithaca
UNITED STATES
mes12@cornell.edu

Sourdille, Pierre
INRA UMR 1095
Genetic, Diversity and Ecophysiology of the Cereals
Avenue du Brázet, 234
63100, Clermont-Ferrand
FRANCE
pierre.sourdille@clermont.inra.fr

Spannagl, Manuel
Helmholtz Center Munich, German Research Center for Environmental Health
Institute of Bioinformatics and Systems Biology
Ingolstadter Landstraie 1
85764, Neuherberg
GERMANY
manuel.spannagl@helmholtz-muenchen.de

Spielmeyer, Wolfgang
CSIRO Plant Industry
GPO Box 1600
Canberra
AUSTRALIA
wolfgang.spielmeyer@csiro.au

Stein, Nils
IPK
Genebank
Corrensstrasse 3
6466, Gatersleben
GERMANY
wahle@ipk-gatersleben.de

Storlie, Eric
INRA
UMR GDEC
234 ave du brezet
63100, Clermont-Ferrand
FRANCE
eric.storlie@clermont.inra.fr

Tayor, Michael
Norwegian University of Life Sciences
Plant and Environmental Sciences
Universitetstunet 3
1432, Ås
NORWAY
tayormichael@yahoo.com

Tiwari, Ratan
Directorate of Wheat Research
Crop Improvement
Agrasain Marg
132 001, Karnal
INDIA
tiwari1964@yahoo.com

Tranquilli, Gabriela
Instituto Nacional de Tecnología Agropecuaria (INTA)
Instituto de Recursos Biológicos (IRB)
N. Repetto y Los Reseros s/n
1686, Hurlingham
ARGENTINA
gtranquilli@cnia.inta.gov.ar

Tyagi, Sandhya
Ch. Charan Singh University
Genetics & Plant Breeding
University Road
250 004, Meerut
INDIA
sandhyatyagi20@yahoo.com

Uauy, Cristobal
John Innes Centre Institution
Department of Crop Genetics
Colney, Norwich
NR4 7UH
UNITED KINGDOM
cristobal.uauy@jic.ac.uk

Valè, Giampiero
CRA-Agriculture Research Council
Genomics research Centre
Via S Protaso 302
29017, Fiorenzuola d'Arda (PC)
ITALY
giampiero.vale@entecra.it

Valárik, Miroslav
Institute of Experimental Botany
Laboratory of Molecular Cytogenetic and Cytometry
Sokolovska 6
77200, Olomouc
CZECH REPUBLIC
valarik@ueb.cas.cz

Vautrin, Sonia
INRA
CNRGV
Chemin de Borde Rouge
31320, Castanet Tolosan
FRANCE
sonia.vautrin@toulouse.inra.fr

Villegas, Dolors
IRTA
Field Crops
Rovira Roure, 191
25198, Lleida
SPAIN
dolors.villegas@irta.cat

Wang, Jiankang
Institute of Crop Science and
CIMMYT China
Chinese Academy of Agricultural Sciences
No. 12 Zhongguancun South Street
100081, Beijing
CHINA
jkwang@cgiar.org

Wang, Jirui
University of California Davis
Department of Plant Science, Luo's Lab
269 Hunt Hall, UCDavis, One Shields Ave
95616, Davis
UNITED STATES
wangjirui@gmail.com

Wang, Xiue
Agriculture College, Nanjing Agricultural University
Weigang 1
210095, Nanjing
CHINA
xiuew@njau.edu.cn

Wanjugi, Humphrey
Monsanto company
Biotechnology
800 N Lindbergh blvd
63167, Saint Louis
UNITED STATES
humphrey.wanjugi@monsanto.com

Waugh, Robbie
The James Hutton Institute**,**
CMS
Errol Road, Invergowrie
DD2 5DA, Dundee
UNITED KINGDOM
Robbie.Waugh@hutton.ac.uk

Webster, Hollie
Murdoch University
Biological Science and Biotechnology
90 South St
6150, Murdoch
AUSTRALIA
hwebster@ccg.murdoch.edu.au

Wenzl, Peter
CIMMYT Int.
GRP
Apdo Postal 6-641
6600, Mexico
MEXICO
p.wenzl@cgiar.org

Wei, Yiqin
Crop Institution of Ningxia Acadamy of Agricultural and Foresty ,China
Crop Institution of Ningxia Acadamy of Agricultural and Foresty Yongning County
750105, Yinchuan
CHINA
yqwei@163.com

Wolters, Petra
DuPont
DuPont Experimental Station
E353 rm 118C
Rt 141 & Henry Clay Rd
19880, Wilmington
UNITED STATES
petra.wolters@cgr.dupont.com

Wright, Jon
The Genome Analysis Centre
Bioinformatics
Norwich Research Park
NR47UH, Norwich
UNITED KINGDOM
Jon.wright@tgac.ac.uk

Wu, Yongzhong
Piioneer Hi-Bred Intl
Research
7250 NW 62nd Ave
50131, Johnston
UNITED STATES
yongzhong.wu@pioneer.com

Yang, Wenlong
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences
Datun Road, Chaoyang District
Beijing
CHINA
wlyang@genetics.ac.cn

Yeo, Freddy
Wageningen University
Plant Breeding
Droevendaalsesteeg 1
6708, PB Wageningen
NETHERLANDS
freddy.yeo@wur.nl

Yousefi, Javan
IMAN
Tuscia University
Department of Science and Technologies for
Agriculture, Forestry,
Nature and Energy
Via s. C. de Lellis,
01100, Viterbo
ITALY
iman.javan@unitus.it

Zakhrabekova, Shakhira
Carlsberg Laboratory
Gamle Carlsberg Vej 10
1799, Copenhagen V
DENMARK
Shakhira.Zakhrabekova@carlsberglab.dk

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International Maize and Wheat
Improvement Center

Apartado Postal 6-641, 06600 Mexico, D.F., Mexico
Website: www.cimmyt.org,
E-mail: cimmyt@cgiar.org