19th International Triticeae Mapping Initiative - 3rd COST Tritigen

Abstracts

August 31st - September 4th 2009

Clermont-Ferrand, FRANCE

19th International Triticeae Mapping Initiative
3rd COST Tritigen

Clermont-Ferrand, France
August 31st – September 4th 2009

Organized by:
INRA-UBP Joint Research Unit Genetics, Diversity and Ecophysiology of Cereals
234 avenue du Brézet
63100 Clermont-Ferrand
France

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In Memoriam

MIKE GALE

1943-2009

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Local Scientific Committee

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Session 6:

A. Murigneux	LIMAGRAIN, Clermont-Ferrand

PROGRAM

MONDAY 31ST AUGUST

8:00am to 9:30am Registration

9:15am to 9:30am Welcome address

- **Michel Beckert** (President of the Clermont-Ferrand Theix INRA center)
- **Catherine Feuillet** (Local Organizing Committee)

9:30am to 10:15am The Mike Gale keynote opening lecture

- **Pat Schnable**, Iowa State University (USA): ***"Maize genomics; new tools and opportunities"***

Session 1: COST WG2 PhysGen, Structural and functional analyses of the Triticeae genomes

10:15am to 11:10am Session 1

Chair: N. Stein

- 10:15am Invited talk: **Eduard Akhunov**, Kansas State University (USA): ***"Using next generation sequencing technology to characterize the gene space of the wheat chromosome 3A"***
- 10:45am **Silvia Fluch**, Department of Health and Environment, Bioresources, PICME, Austrian Institute of Technology (Austria): ***"Sequence composition of the 1RS rye (Secale cereale) chromosome arm present in Triticum as revealed by 454 FLX sequencing"***

Coffee break

11:30am to 12:30pm Session 1

Chair: N. Stein

- 11:30am **Eva Bauer**, Technische Universität München, Plant Breeding (Germany): ***"GABI RYE-EXPRESS: Unlocking the genetic potential of rye by establishing a functional genomics resource for the EXPRESSed portion of the rye genome"***
- 11:50am **Edwin van der Vossen**, Keygene (Netherland): ***"Application of CRoPS technology in durum wheat: SNP discovery and subsequent mapping in a multiparental crossing"***
- 12:10am **Camille Rustenholz**, INRA-UBP UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales (France): ***"Impact of the genome structure on the gene space organisation, function and regulation of wheat chromosome 3B"***

Lunch

2:15pm to 3:30pm Session 1

Chair: P. Sourdille

- 2:15pm Invited talk: **Robbie Waugh**, SCRI (UK)-COST: ***"Whole genome scans in elite barley germplasm as a strategy for gene isolation"***
- 2:45pm **Cédric Moisy**, Biotechnology and Food Research, MTT Agrifood Research Finland (Finland): ***"Towards High-Throughput Transposable Element Markers for Barley"***
- 3:05pm **Nils Stein**, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (Germany): ***"Sequencing the barley genome accelerated by Next-Generation Sequencing Technology"***

Session 2: COST WG1 Divgen, Exploring and exploiting genetic diversity of the Triticeae

3:30pm to 4:20pm Session 2

Chair: F. Balfourier

- 3:30pm Invited talk: **Michael McKay**, Bioversity International (Italy): ***"Towards more efficient mining of genetic variation in ex situ collections"***
- 4:00pm **Konstantin (Kostya) Kanyuka**, Department of Plant Pathology and Microbiology, Rothamsted Research (United Kingdom): ***"A new rapid and relatively inexpensive method for mining in plant germplasm collections for novel alleles of agronomically important genes: a case study in barley"***

 ***Coffee break***

4:50pm to 6:20pm Session 2

Chair: T. Fahima

- 4:50pm **Andreas Börner**, Leibniz Institute of Plant Genetics and Crop Plant Research (Germany): ***"The preservation and exploitation of ex situ genebank collections – Association mapping studies in wheat"***
- 5:10pm **Susanne Dreisigacker**, CIMMYT (Mexico): ***"Haplotyping the history of CIMMYT International Nurseries in wheat"***
- 5:30pm **Guangmin Xia**, School of Life Science, Shandong University (China): ***"A Salt Responsive Gene Di19A in wheat somatic hybrid introgression line"***
- 5:50pm **Mehmet Cakir**, Murdoch University (Australia): ***"A global effort in germplasm characterization and breeding for resistance to Russian wheat aphid in Wheat and Barley"***

7:00pm Welcome Reception at the City Hall in Clermont-Ferrand

TUESDAY 1ST SEPTEMBER

Session 3: COST WG3 Traitgen, Deciphering agronomical traits and phenotypes in the Triticeae

9:00am to 10:30am Session 3: Yield

Chair: R. Tuberosa

- 9:00am Invited talk: **Jérôme Salse**, NRA-UBP UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales (France)-COST: ***"Cross Genome Map-based Cloning of a nitrogen use efficiency meta-QTL on wheat chromosome 3B unravels new evidence for cereal genome evolution"***
- 9:30am **Hirokazu Handa**, Graduate School of Life and Environmental Sciences, University of Tsukuba (Japan): ***"Functional diversification of barley FT-like genes in flowering"***
- 9:50am **Delphine Capron**, UMR GDEC 1095 INRA Université Blaise Pascal (France): ***"Expression profiling of the Skp1-Cullin-F-box (SCF) E3 ligases during wheat grain development"***
- 10:10am **Marion Röder**, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (Germany): ***"Genetic Dissection of a QTL for Grain Size in Wheat"***

Coffee break

11:00am to 12:30pm Session 3: Abiotic Stresses

Chair: D. Habash

- 11:00am Invited talk: **Peter Langridge**, ACPFG (Australia): ***"Genomics of stress tolerance in low yielding environments"***
- 11:30am **Nicholas Collins**, Australian Centre for Plant Functional Genomics, School of Agriculture Food and Wine, University of Adelaide, (Australia): ***"Reproductive frost tolerance genes in barley"***
- 11:50am **Ana Casas**, Aula Dei Experimental Station (EEAD-CSIC) (Spain): ***"Allelic effects of some photoperiod and vernalization barley genes on flowering time under Mediterranean conditions"***
- 12:10pm **Thorsten Schnurbusch**, Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Genebank Department (Germany): **"Lower transcript levels of HvNIP2;1 at the Bot3 locus in barley confer increased tolerance to high boron supply"**

Lunch

2:00pm to 3:30pm Session 3: Biotic Stresses

Chair: R. Niks

- 2:00pm Invited talk: **Beat Keller**, Institute of Plant Biology (Switzerland): ***"Pm3 and Pm8 based powdery mildew resistance in wheat and rye: a molecular analysis"***
- 2:30pm **Sylvie Cloutier**, CRC-AAF Canada, Winipeg (Canada): ***"Expression profiling of the wheat leaf rust Lr1 pathosystem using transgenic lines and the Affymetrix wheat chip"***
- 2:50pm **Patrick Schweizer**, Leibniz Institute of Plant Genetics and Crop Plant Research IPK (Germany): ***"Convergent evidence for genes underlying quantitive pathogen resistance in Barley"***
- 3:10pm **Thierry C. Marcel**, UMR 1290 INRA AgroParisTech BIOGER-CPP (France): ***"A map-based cloning approach to unravel genes for basal resistance to biotrophic fungi in barley"***

Coffee break

4:00pm to 6:00pm Parallel Events

- 1st Poster session *(Sessions 1 and 2)*
- IBSC Business meeting
- IWGSC coordinating committee meeting

WEDNESDAY 2ND SEPTEMBER

Session 3: COST WG3 Traitgen, Deciphering agronomical traits and phenotypes in the Triticeae

9:00am to 10:30am Session 3: Quality

Chair: R. Appels

- 9:00am Invited talk: **Domenico Lafandria**, Universita degli Studi della Tuscia (Italy): ***"Modifying protein and starch composition of wheat kernel for technological and nutritional improvement"***
- 9:30am **Agostino Fricano**, Parco Tecnologico Padano (ITALY): ***"Mapping Quality Traits Associated with Grain Micronutrient Content in Diploid Wheats Using Interspecific Introgression Lines"***
- 9:50am **Jacques Le Gouis**, INRA-UBP UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales (France): ***"Multi-environment QTL mixed models for dissecting N use efficiency and tolerance to low N in wheat"***
- 10:10am **Vidya Gupta**, PMB Unit, Biochemical Sciences Division, National Chemical Laboratory (India): ***"Molecular Dissection of Breadmaking Quality and Kernel Characters in Wheat"***

Coffee break

11:00am to 12:30pm Parallel Events

- 2nd Poster session *(Sessions 3 to 7)*
- TriticeaeGenome ExCommittee meeting

Lunch

2:00pm Departure for social activities and Gala Dinner

The bus will take you back to the Hotels at the end of the Gala Dinner

THURSDAY 3RD SEPTEMBER

Session 4: Genome Structure and Evolution

9:00am to 10:30am Session 4

Chair: K. Devos

- 9:00am Invited talk: **Xue-Yong Zhang**, CAAS Beijing (China): ***"Structure and evolution of wheat centromeres and pericentromeres"***
- 9:30am **Frédéric Choulet**, INRA-UBP UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales (France): ***"Sequencing, annotation and characterization of 17 Mb of chromosome 3B contigs provide novel insights into the wheat genome organization and evolution"***
- 9:50am **Elena Salina**, Institute Cytology and Genetics (Russia): ***"Structural analysis of subtelomeric chromosomal region of Triticum and Aegilops species"***
- 10:10am **Jasdeep Mutti**, Washington State University (USA): ***"Gene Expression Balance Among Homoeologues And Its Interdependence On Gene Dosage In Polyploid Wheat"***

Coffee break

11:00am to 12:30pm Session 4

Chair: E. Paux

- 11:00am Invited talk: **Thomas Wicker**, University of Zurich (Switzerland)-COST: ***"Studies on genome organisation and evolution in the age of high throughput sequencing: So much data and so little time!"***
- 11:30am **Boulos Chalhoub**, CEA: Institut de Génomique (France): ***"Dynamics and differential proliferation of transposable elements in the wheat genomes"***
- 11:50am **Sunish Kumar Sehgal**, Department of Plant Pathology (USA): ***"Sequence based comparison of Mega-base homoeologous regions of A and B genomes of Bread wheat"***
- 12:10pm **Andy Flavell**, University of Dundee at SCRI (UK): ***"Genetic diversity analysis of wild and landrace barleys using the BOPA1 Illumina genotyping platform"***

Lunch

Session 5: COST WG2A Bioinformatics, Data management and integration

2:00pm to 3:30pm Session 5

Chair: A. Schulman

- 2:00pm Invited talk: **Klaus Mayer**, MIPS/IBIS, Helmholtz Center Munich (Germany): ***"Gramene: A database for comparative plant genomics"***
- 2:30pm **David Matthews**, USDA-ARS, Dept. of Plant Breeding and Genetics (USA): ***"GrainGenes, the Triticeae Genome Database"***
- 2:50pm **Timothée Flutre**, INRA (UR 1164) Unité de Recherche en Génomique-Info (France): ***"REPET: pipelines for the identification and annotation of transposable elements in genomic sequences"***
- 3:10pm **Tsuyoshi Tanaka**, National Institute of Agrobiological Sciences (Japan): ***"Towards annotating Triticeae genomes by cross-species cDNA mapping"***

Coffee break

4:00pm to 5:10pm Session 5

Chair: H. Quesneville

- 4:00pm Invited talk: **Patrick Wincker**, Genoscope (France): ***"Perspectives on using new high throughput sequencing technologies on decoding the genomes of plant species"***
- 4:30pm **Philippe Leroy**, INRA-UBP UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales (France): ***"TriAnnot: an automatic pipeline for Triticeae genome annotation"***
- 4:50pm **Michael Alaux**, INRA (UR 1164) Unité de Recherche en Génomique-Info (FRANCE): ***"Wheat data on GnpIS, the INRA URGI information system"***

5:10pm to 7:00pm Parallel Events

- Technological advances presented by companies:

Chair: H .Bergès

- **Gregory Penner**, NeoVentures Biotechnology Inc. (Canada): ***"High throughput proteomics for SNP identification within breeding programs"***
- ABI (France): ***"SOLiD™ Sytem for plant genomics"***
- **Céline Capéra**, Beckman Coulter Genomics (France): ***"New solutions for plant genomics"***

- COST action Tritigen Management ExCommittee meeting

FRIDAY 4TH SEPTEMBER

Session 6: Molecular breeding

9:00am to 10:30am Session 6

Chair: V. Korzun

- 9:00am Invited talk: **David Bonnett**, CIMMYT (Mexico): ***"Wheat Molecular Breeding and Pre-breeding at CIMMYT"***
- 9:30am **Kerstin Hofmann**, Bavarian State Research Centre for Agriculture, Institute for Crop Science and Plantbreeding 1b (Germany): ***"Rhynchosporium secalis resistance in barley - from mapping to marker development and pre-breeding material"***
- 9:50am **Borislav Kobiljski**, Institute of Field and Vegetable Crops (Serbia): ***"The validation and use of marker-assisted selection in NS wheat breeding program"***
- 10:10am **Walid Alfares**, INRA-UBP UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales (France): ***"Fine mapping and marker development for the crossability gene SKr on chromosome 5BS of hexaploid wheat (*Triticum aestivum L.*)"***

Coffee break

11:00am to 12:15pm Session 6

Chair: P. Perez

- 11:00am Invited talk: **Sebastien Praud**, Biogemma/LVH (France): ***"The future impact of genomics assisted approaches in maize breeding"***
- 11:30am **Victor Korzun**, KWS LOCHOW GMBH (Germany): ***"Application of molecular marker in cereals breeding"***
- 11:50am **Marcel De Leeuw**, Cogenics (France): ***"High throughput SNP discovery in wheat using methylation-sensitive digestion and next-generation sequencing"***

Lunch

Session 7: Focused groups

2:00pm to 3:30pm Session 7

Chair: C. Feuillet

- International Wheat Genome Sequencing Consortium Activity report: **K. Eversole**
- International Barley Sequencing Consortium Activity report: **N. Stein**
- COST action FA0604 Tritigen Activity report: **A. Schulman**
- International wheat SNP working group Activity report: **E. Akhunov**

3:30pm to 4:00pm

- Concluding Remarks (**C. Feuillet**)
- Announcement of the next ITMI workshop (**J. Jizeng**)

4:00pm End of the conference

Oral and poster presentations

PRESENTATIONS

Oral presentations

Abstract reference: ITMI2009_176

THE MIKE GALE KEYNOTE OPENING LECTURE

Maize Genomics: New Tools and Opportunities

Schnable Patrick[1], Swanson-Wagner Ru[1], DeCook Rhonda[2], Jia Yi[1], Ji Tieming[1], Zhao Xuefeng[1], Nettleton Dan[1], Ying Kai[1], Iniguez A. Leonardo[3], Rosenbaum Heidi[3], Yeh Eddy[1], Kitzman Jacob[3], Richmond Todd[3], Ji Tieming[1], Wu Wei[1], Barbazuk Brad[4], Jeddeloh Jeff[3], Springer Nathan[5], Fu Yan[1], and the Maize Genome Sequencing Project[1,6,7,8]

[1]Iowa State University NA 50011 Ames IA USA, [2]University of Iowa NA 52242 Iowa City, IA USA, [3]NimbleGen 1 Science Court 53711 Madison, WI USA, [4]University of Florida NA 32611 Gainesville, FL USA, [5]University of Minnesota NA 55113 Minneapolis, MN USA, [6]Washington University NA 63130 St. Louis, MO USA, [7]Cold Spring Harbor Laboratory One Bungtown Road 11724 Cold Spring Harbor, NY USA, [8]University of Arizona NA 85721 Tucson, AZ USA

A project to generate a near-complete sequence of the maize inbred B73 is utilizing a minimal tiling path of approximately 16,000 mapped BAC clones. The project is focusing on producing high-quality sequence coverage of the genic regions which are being ordered, oriented and anchored to physical and genetic maps.

Following its domestication ~10,000 years ago, breeders have exploited the extensive genetic diversity of maize. The roles of structural variation, including insertions, deletions and copy number variation on the phenotypic diversity and plasticity of this important crop have not been elucidated. Whole-genome array-based comparative genomic hybridizations (aCGH) revealed that structural variation between the B73 and Mo17 inbreds is not evenly distributed across the genome. Analysis of altered segments of DNA identified hundreds of sequences that exhibit copy number variation among the two genotypes, as well as thousands of sequences that are present in B73 but not Mo17. Sequences present in B73, but not Mo17, genome include full-length genes, gene fragments and transposons.

Although widely exploited in agriculture, the mechanisms responsible for heterosis are not well understood. Being monoecious it is possible to use a given maize plant as both male and female parents of crosses. Regardless of cross direction, the inbred lines B73 and Mo17 produce heterotic hybrids. These reciprocal hybrids differ phenotypically from each other despite having identical nuclear genomes. Consistent with these phenotypic observations, thousands of differentially expressed genes were detected between the reciprocal hybrids. An eQTL experiment conducted to better understand the regulation of gene expression in inbred and hybrid lines detected over ~4,000 eQTL associations. The bulk of eQTL act *in trans* to regulate expression of genes on other chromosomes. Surprisingly, for many of the trans-eQTL, heterozygous eQTL differentially regulate transcript accumulation in a manner consistent with gene expression in the hybrid being regulated exclusively by the paternally transmitted allele. Because the designs of these experiments control for cytoplasmic and maternal effects, these findings suggest that widespread parent-of-origin effects may contribute to the phenotypic differences between reciprocal hybrids.

Session 1:
COST WG2 PhysGen, Structural and functional analyses of the Triticeae genomes

Abstract reference: ITMI2009_169

Using next-generation sequencing technology to characterize the gene space of the wheat chromosome 3A

Akhunova Alina[1], Catana Vasile[1], Sehgal Sunish Kumar[1], Dolezel Jaroslav[2], Simkova Hana[2], Kubalakova Marie[2], Gill Bikram[1], Akhunov Eduard[1]

[1]Kansas State University 4024 Throckmorton Plant Sciences Center 66502 Manhattan USA, [2]Institute of Experimental Botany Sokolovska 6 CZ-77200 Olomouc Czech Republic

The wheat genome sequencing is complicated by its large size, polyploidy and high repetitive DNA content. A number of experimental approaches have been developed to overcome these complications. One of these approaches, the flow-sorting of mitotic chromosomes, was shown to be very efficient for the dissection and analysis of complex plant genomes. It has been successfully used for the construction of chromosome-specific BAC libraries, targeted development of molecular markers and integration of genetic and physical maps. We used the flow-sorted chromosome arms to perform shotgun sequencing and characterization of the gene space of the wheat chromosome 3A. Both ABI's SOLiD and Roche's 454 next-generation sequencing platforms have been selected to produce sequence data equivalent to 10X chromosome coverage. The hybrid sequencing approach including a combination of 454 single and mate-paired reads with SOLiD mate-paired reads is being used to generate the first draft assembly of the wheat chromosome 3A. The utility of various computational algorithms for the assembly of hybrid sequence data is being tested. The results of shotgun sequence assembly and functional and structural annotation of genes located on the wheat chromosome 3A will be presented.

Abstract reference: ITMI2009_087

Sequence composition of the 1RS rye (Secale cereale) chromosome arm present in Triticum as revealed by 454 FLX sequencing

Fluch Silvia[1], Kopecky Dieter[1], Berenyi Maria[1], Simkova Hana[3], Klauninger Bert[1], Suchankova Pavla[3], Lelley Tamas[2], Taudien Stefan[4], Platzer Matthias[4], Doležel Jaroslav[3], Burg Kornel[1]

[1]Department of Health and Environment, Bioresources, PICME, Austrian Institute of Technology - 2444 Seibersdorf Austria, [2]Institute of Biotechnology in Plant Production, Department of Agrobiotechnology IFA-Tulln, Univ. of Natural Resources and Applied Life Sciences (BOKU) Konrad Lorenz Strasse 20 3430 Tulln Austria, [3]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany Sokolovska 6 77200 Olomuc Czech Republic, [4]Genome Analysis, Leibniz Institute for Age Research, Fritz Lipmann Institute (FLI) Beutenbergstraße 11 07745 Jena Germany

Rye (*Secale cereale* L.) is a member of the wheat tribe (Triticeae) and is grown extensively as a grain and forage crop. Numerous known and unknown wheat lines worldwide are carrying the 1BL.1RS wheat-rye chromosome translocation making the short arm of rye chromosome 1 (1RS) an integrated part of the wheat germplasm conferring high value agronomic traits to wheat. 1RS is present as a pair of telocentric chromosomes in Chinese Spring/Imperial ditelo1RS wheat/rye addition line allowing the isolation of this chromosome by flow cytometric sorting. Recently, a protocol for multiple displacement amplification (MDA) of DNA from flow-sorted chromosomes has been developed, expanding range of applications of flow sorted chromosomes in plant genomics. MDA amplification is representative and produces microgram amounts of DNA from only about 10,000 chromosomes. In this study, DNA amplified from flow sorted chromosome 1RS has been subjected to 454 sequencing (GS FLX, Roche) resulting in 235Mbp of sequence data, yielding about 0.5x coverage of the 441Mbp chromosome arm. Comparative analysis using the TREP and MIPS repeat databases revealed that 76% of the obtained sequences represented known transposon elements predominated by Class1 retro elements (62%), which were mostly of Gypsy type (52%). Class2 DNA transposons were represented by only 10% of the sequences, and were mainly of CACTA type (8%). As far as genes were concerned, the ribosomal locus was represented by 2% of the sequences and the Secalin gene family was also represented by numerous sequence reads. Additionally putative telomere and centromere specific sequences could also be detected. The detailed analysis of the study will be presented.

Abstract reference: ITMI2009_114

GABI RYE-EXPRESS: Unlocking the genetic potential of rye by establishing a functional genomics resource for the EXPRESSed portion of the rye genome

Bauer Eva[1], Haseneyer Grit[1], Schmutzer Thomas[2], Seidel Michael[3], Schön Chris-Carolin[1], Mayer Klaus[3], Scholz Uwe[2], Stein Nils[4]

[1]Technische Universität München, Plant Breeding Am Hochanger 4 85350 Freising Germany, [2]Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Bioinformatics and Information Technology Corrensstr. 3 06466 Gatersleben Germany, [3]Helmholtz Zentrum München, Munich Information Centre for Protein Sequences (MIPS), Ingolstädter Landstraße 1, 85764 Neuherberg, Germany, [4]Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Genome Diversity Research Group Corrensstr. 3 06466 Gatersleben Germany

Rye (*Secale cereale* L.) exhibits a high tolerance to abiotic stresses (drought, frost, low soil fertility) but missing sequence information of the rye genome hampers the systematic exploration of the genetic potential for accelerated cultivar improvement. The main emphasis of the project is to unlock the genetic potential of rye by establishing a functional genomics resource for the EXPRESSed portion of the rye genome.

Five highly diverse rye inbred lines were used for cDNA sequencing. To capture a comprehensive part of the rye transcriptome, RNA of each genotype was obtained from a set of plant tissues and developmental stages. cDNAs were pooled per genotype, normalized and sequenced on a Roche/454 GS FLX platform. After the adaptor removal, more than 2.6 Mio. sequence reads with a median length of 216 bp could be entered into the EST assembly process. Based on a preliminary sequence comparison (BLASTN) the majority (>80%) of the reads revealed sequence similarity to other cereal sequences.

After accomplishment and evaluation of the EST assembly a robust set of unigenes shared among all genotype-specific sequence datasets will be delivered and utilized for in silico SNP mining. The development of an automated SNP detection pipeline combining publicly available SNP detection tools and project related features is under development. A set of relevant parameters was defined and will be implemented to avoid (i) assembly of paralogous sequences, (ii) misassembly due to presence of repetitive DNA and (iii) SNP detection within homopolymer stretches. The goal is to develop a high-throughput genotyping platform for SNPs on the basis of an Illumina Golden Gate Assay. Within the scope of this project three rye mapping populations (set A), a diversity panel of inbred lines representing the available rye genepools, parents of additional mapping populations and the material used in GABI RYE-FROST (set B) will be genotyped. Data obtained from set A will be used to generate a high-density rye transcript map. The map will be instrumental to assess colinearity of the rye genome to other grass genome resources. Genotyping data from set B will be used for estimating intra- and inter-chromosomal linkage disequilibrium within GABI RYE-FROST and for the assessment of genetic diversity parameters.

The current status of the sequence assembly and SNP detection will be presented.

Abstract reference: ITMI2009_160

Application of CRoPS technology in durum wheat: SNP discovery and subsequent mapping in a multiparental crossing

van der Vossen Edwin[1], Trebbi Daniele[2], Maccaferri Marco[3], Sørensen Anker[1], Giuliani Silvia[3], Sanguineti Maria Corinna[3], Massi Andrea[4], Tuberosa Roberto[3]

[1]Keygene N.V. Agro Business Park 90 6700 AE Wageningen The Netherlands, [2]Institute of Applied Genomics IGA Via J.Linussio 51 33100 Udine Italy, [3]Dept. Agroenvironmental Science and Technology DiSTA Viale Fanin 44 40127 Bologna Italy, [4]Società Produttori Sementi Bologna PSB via Macero 1 40050 Argelato Italy

Single nucleotide polymorphism (SNP) platforms are of great value for high-throughput molecular profiling and haplotype-based approaches. In the EU-funded project BIOEXPLOIT, we have undertaken the development of an SNP platform in durum wheat based on the Complexity Reduction of Polymorphic Sequences (CRoPS^{TM}) method developed at Keygene. CRoPS is based on the combination of a genome complexity reduction method (AFLP) and highly parallel sequencing with Roche's Genome Sequencer FLX. Because multiparental inbred populations have been proposed as a more efficient alternative to classical bi-parental populations, we have established a four way-cross recombinant inbred (RI) mapping population in durum wheat by using four highly diverse cultivars (Neodur, Claudio, Colosseo and Rascon/Tarro) carrying useful alleles for disease resistance, quality and yield. Presently, 90 four-way F_1 plants have been genotyped for genetic map development, while seed for the final RIL population (380 F_8 RILs) will be available in June 2009. SSR and AFLP markers (189 AFLPs and 70 SSRs) were mapped on 22 linkage groups, spanning a total of 1080 cM. To increase the map density and coverage, CRoPS analysis on the four parental varieties identified more than a thousand putative polymorphic SNP loci (from 589,826 sequences with an average length of 155 bp). These putative SNPs have been used to develop two Illumina based BeadExpress assays that each interrogate 384 putative SNP loci in a multiplex manner. Work is in progress to profile the F_8 RILs with the Illumina SNP platform. The integration of the current map with the SNP markers will improve the quality and coverage of the genetic map.

Abstract reference: ITMI2009_009

Impact of the genome structure on the gene space organisation, function and regulation of wheat chromosome 3B

Rustenholz Camille[1], Choulet Frédéric[1], Hedley Pete[2], Waugh Robbie[2], Feuillet Catherine[1], Paux Etienne[1]

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Because of its size (17Gb), allohexaploid nature and high repeat content (>80%), the wheat genome has always been perceived as too complex for efficient molecular studies. As a consequence, our knowledge of the wheat genome structure is still very limited. In particular, the impact of the genome structure on the organisation, function and regulation of genes at a chromosome scale is poorly understood. Following a chromosome-specific approach, we recently constructed the physical map of the chromosome 3B. This map allowed for the development of unique genomic resources that can be used to decipher the structure, evolution and function of the wheat genome. Based on these novel resources, we applied transcriptomics approaches to get new insights into relationships between structure of the genome, function and regulation of the genes along wheat chromosomes.

First, chromosome 3B minimal tiling path (MTP) BAC arrays were hybridized with mRNA extracted from five wheat organs at three developmental stages each to identify gene-containing BACs. Then, barley Agilent 15K expression microarrays were hybridized with the 3D MTP pools to locate precisely genes on the physical map. These experiments resulted in complementary conclusions shedding light on the chromosome 3B gene space organisation. They showed that genes are distributed throughout the chromosome and not limited to distal regions. Moreover, no megabase-sized "geneless" region was identified suggesting that no large genomic regions are completely devoid of genes. These results are consistent with gene distribution observed at the sequence level through the sequencing and annotation of 12 large contigs (cumulative length of 15 Mb) representing different regions of the chromosome 3B. Deletion bin-mapping of genes revealed an uneven distribution of genes along the chromosome 3B, with a gradient of gene density from centromere to telomeres.

To further investigate gene space organisation and regulation, hybridizations are currently carried out on wheat NimbleGen 45K expression chips to establish fine expression patterns and construct a transcriptional map of the chromosome 3B. At the gene scale, the impact of the genome structure on homoeologous gene regulation is investigated through transcript profiling of genes showing contrasting structural context.

Altogether, the data obtained will help deciphering the relationships between the hexaploid wheat genome structure and the function and regulation of genes.

Abstract reference: ITMI2009_181

Whole-genome association mapping in elite inbred crop varieties

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In the early 2000's we set out to investigate the potential of using association mapping to genetically dissect phenotypic traits in the large genome, small grain cereal crop plant, barley. At that time we had no idea of the extent of LD in different genepools, used anonymous molecular marker techniques, knew little about population structure (or how to deal with it) and had no guidelines about what to expect from LD-based mapping in an inbreeding crop. However, LD-mapping was attractive for a number of reasons, not least being the potential to exploit already available historical trait data collected during the 'National' and 'Recommended' list trialling processes, and concerns from the barley breeding community that the bi-parental experimental populations commonly worked on by academics had little relevance to the material currently used in crop improvement. We therefore set about to systematically assemble the tools, resources and understanding that would allow us to explore the potential, and ultimately apply, association mapping in barley. In this presentation I will summarise the route we have taken to bring our original vision to fruition and some of the successes we have recently achieved in identifying genes underlying specific phenotypic traits.

Abstract reference: ITMI2009_076

Towards High-Throughput Transposable Element Markers for Barley

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Molecular markers are playing an increasingly important role in plant breeding, including marker-based selection. The ubiquity, abundance, and genomic dispersion of transposable elements have made them excellent sources of molecular markers. In addition, the mobility of transposable elements has made it possible in several crops to distinguish closely related cultivars even when other marker methods cannot. In addition, the prevalence of transposable elements within the genome tends to vary inversely with gene density, although they are seldom absent from gene-rich regions. These properties of transposable elements provide a niche for marker systems based on them. For example, assembly of physical maps and their linkage to genetic maps needs markers that help span gene-poor segments of the genome, where component BAC clones may be fully lacking in genes. In order to build a physical map of barley and to facilitate the assembly of the genome sequences within the EU project Triticeae Genome, we are developing high-throughput marker methods based on transposable elements insertions in barley. We are focusing on the Miniature Inverted-repeat Transposable Elements (MITEs). These are structurally similar to defective Class II elements, the "cut-and-paste" DNA transposons, but their high copy numbers suggest that they can be amplified by a replicative mechanism. Using bioinformatics tools and MITEs available in the TREP database, we have first identified MITEs in barley BAC-end Sequences and designed consensus primers for families of short MITEs of high copy number. They have served for the production of probes for the capture of genomic MITE sequences for the high-throughput sequencing of the MITEs and their flanks. Polymorphic MITEs can now be used both for high-throughput scoring of insertion site polymorphisms and for linkage of BACs containing these MITE loci to the genetic map.

Abstract reference: ITMI2009_101

Sequencing the barley genome accelerated by Next-Generation Sequencing Technology

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Although significant molecular resources have been established for barley over the past decade, efficient gene isolation or studies of genome-wide diversity are hampered due to the lack of access to a high quality reference genome sequence. Under the auspices of the International Barley Genome Sequencing Consortium (IBSC, http://barleygenome.org) efforts are underway to build a physical map as a tool for gene isolation but furthermore to serve as the template for a clone-based strategy of sequencing the barley genome.

The introduction of next generation sequencing (NGS) technology promises a significant reduction of overall sequencing costs – a basic prerequisite for making large crop genome sequencing a thinkable task. However, read length which is an important feature for bridging repetitive DNA populations in the Triticeae genomes is a limitation characteristic to these new methods. We employ the Roche 454 GSFLX and Illumina/Solexa platforms for different tasks of genomic sequencing in barley. Over 1500 of a planned total of >3000 BAC (~5% of the barley genome) clones have been sequenced by a barcoded BAC pool sequencing approach on GSFLX and the assembly results provided proof of the usefulness of the approach. Whole genome shotgun sequencing of barley genomic DNA on the Illumina/Solexa 1G delivered straightforward sequence information for masking repetitive DNA during the annotation process. 454 shotgun sequencing of flow-sorted barley chromosomes provides access to gene indices for entire chromosomes and allows to design virtual gene orders of barley chromosomes by consulting synteny to sequenced grass genomes like those of rice, sorghum, Brachypodium and maize. In summary, access to NGS technology has changed completely the way of Triticeae genome sequencing – in fact they build the basis for entering into comprehensive endeavors.

Session 2:
COST WG1 Divgen, Exploring and exploiting genetic diversity of the Triticeae

Abstract reference: ITMI2009_174

Towards more efficient mining of genetic variation in ex situ collections

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The efficient and effective use of *ex situ* collections of plant genetic resources for food and agriculture (PGRFA) is necessitated by the challenges facing modern plant improvement. Historically, finding the necessary allelic variation amongst the millions of accessions in genebanks has often been a combination of researcher intuition, availability of accessions, previous experience and chance. A number of approaches have been put forward to improve the utilization of PGRFA without making much of an impact on either effectiveness or efficiency. The Focused Identification of Germplasm Strategy (FIGS) is a heuristic process that has demonstrated considerable impact, both in terms of efficiency and effectiveness, in identifying 'candidate' accessions for specific adaptive traits. The logic behind the strategy is presented together with several examples of how it has been successfully used to explore and exploit PGRFA.

Abstract reference: ITMI2009_049

A new rapid and relatively inexpensive method for mining in plant germplasm collections for novel alleles of agronomically important genes: a case study in barley

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The plant eIF4E (eukaryotic translation initiation factor 4E) plays an important role in the life cycle of viruses belonging to the family *Potyviridae*, including bymoviruses BaMMV and BaYMV. A physical interaction between eIF4E and the viral protein VPg is essential for virus multiplication. It has been recently demonstrated that in barley two allelic recessive genes *rym4* and *rym5* providing resistance to bymoviruses encode variants of eIF4E, which are likely to be incapable of binding VPg. The aim of our current study was to explore the full extent of variation of *eIF4E* available in the natural germplasm, and to identify novel alleles potentially providing broad-spectrum resistance to bymoviruses. A selection of > 1100 barley accessions (primarily landraces and old barley cultivars) from all major barley growing regions of the world was assembled. The coding region of *eIF4E* was analysed in ~ half of this barley collection using direct sequencing of amplified *eIF4E* cDNA. For the analysis of the other half of the collection we developed a different, two-step approach. In the first step, the PCR amplicons derived from *eIF4E* cDNA were pre-screened for the presence of DNA polymorphisms using a high-resolution melting (HRM), which is a simple and non-destructive close-tube assay. In the second step, only the cDNA amplicons predicted using HRM to carry SNPs were sequenced. This new approach proved to be rapid, economical, and reliable for the detection of SNPs and small deletions and insertions in the coding sequence of *eIF4E*. In total, more than 40 novel alleles were identified in our barley diversity collection. The majority of these contained non-synonymous nucleotide substitutions, which resulted in amino acid changes in the encoded protein. Interestingly, the highest diversity of *eIF4E* alleles appeared to occur in geographic regions with a history of yellow mosaic disease. This may suggest a possible co-evolution race between barley and bymoviruses and this will be investigated in the future work. The 3-D protein modelling revealed that most of the identified amino acid changes map to a specific region near to the cap-binding pocket of eIF4E suggesting a potential role of this region in interaction with VPg. Accessions carrying the newly identified *eIF4E* alleles are being subjected to bioassays with five European bymovirus isolates. Preliminary experiments suggest most of the novel *eIF4E* alleles in barley direct resistance to one or more bymovirus isolates.

Abstract reference: ITMI2009_069

The preservation and exploitation of ex situ genebank collections – Association mapping studies in wheat

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As estimated by FAO world-wide existing germplasm collections contain more than 6 million accessions of plant genetic resources. Wheat (*Triticum* and *Aegilops*) represents the biggest group with about 800,000 accessions. One of the four largest *ex situ* genebanks of the world is located at the Leibniz-Institute of Plant Genetics and Crop Plant Research in Gatersleben. As on the global scale wheat is the largest group having almost 30,000 accessions. Beside the long term storage and frequent regeneration of the material phenotypic characterisation and evaluation data are collected as a prerequisite for gene identification and mapping. In our presentation we demonstrate the successful utilisation of a germplasm collection for the identification and molecular mapping genes (QTLs) determining agronomic important traits, exploiting an association-based approach. Here a larger population of individual genotypes is analysed in order to detect associations between marker patterns and trait expressions.
A genome wide association analysis was performed using a genetically diverse core collection of 96 wheat accessions. The genotypes were evaluated for agronomic traits during up to five growing seasons. These traits include heading date, plant height as well as several yield determining parameters as for example thousand grain weight but also diseases. In order to investigate trait-marker associations the wheat lines were genotyped using 874 diversity array technology (DArT) markers. For investigation of the population structure a subset of 219 markers was analysed with the programme STRUCTURE. It revealed a structure of two possible subpopulations, what can be explained by the origin and pedigrees of the material. The analyses of associations between markers and traits were performed with TASSEL using the General Linear Model with the Q-Matrix received from STRUCTURE as correction for the population structure. Several main and minor associations for all investigated traits were obtained and are presented. Homologous and homoeologous relationships of detected loci are discussed.

Abstract reference: ITMI2009_099

Haplotyping the history of CIMMYT International Nurseries in wheat

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International nurseries are specialized experimental plots for advanced breeding lines and are coordinated by international agricultural research centers within the Consultative Group of International Agricultural Research (CGIAR). Distributed and grown by many national research programs and participants worldwide the international nurseries serve as springboards for the development of new crop varieties. They have become indispensable for the research centers to screen their elite breeding material for wide adaptability as well as for resistance to specific disease pressure. At the International Center for Maize and Wheat Improvement (CIMMYT) international nurseries have a considerable history, the first nursery being distributed in 1960s. Today 40 different nurseries are prepared every year and received by approximately 120 countries.

While the nurseries describe the phenotype of CIMMYT wheat germplasm across many different environments in the world, the genotype of the germplasm has not been characterized yet. CIMMYT international nurseries are currently described with genome wide scans and diagnostic DNA markers for various traits. Results will be presented.

Abstract reference: ITMI2009_140

A Salt Responsive Gene Di19A in wheat somatic hybrid introgression line

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A new somatic hybrid introgression line Shanrong No.3 (SR3) has been generated in our lab from hybridization of common wheat Jinan 177 (JN177) with *Thinopyrum ponticum*, a salt and drought tolerant grass. Cytological and molecular analysis showed that some nuclear and non-nuclear DNAs and even functional genes of donor *T. ponticum* were introgressed into this line. SR3 had a significantly higher yield than its parent JN177 in salt-alkali soil of Shandong, China. It has passed Shandong provincial regional yield trial for new salt-enduring wheat cultivar (Lu-Nong-Shen-Zi No. [2004]030). Based on the differential screening of SSH cDNA library, *TaDi19A* was clone from SR3, which belonged to Di19 (DROUGHT INDUCED 19) gene family. *TaDi19A* was localized in long arm of the chromosome 3B and its protein products mainly presented in nucleus. It was constitutively expressed in both the roots and leaves of wheat seedlings grown under non-stressed conditions, but was substantially up-regulated by the imposition of stress (salinity, drought and cold), or the supply of stress-related hormones (ABA and ethylene). The heterologous over-expression of *TaDi19A* in *Arabidopsis thaliana* increased the plants' sensitivity to salinity stress, ABA and mannitol during the germination stage. Root elongation in these transgenic lines showed a reduced tolerance to salinity stress and a reduced sensitivity to ethophon. Flowering was accelerated in the transgenic lines when stressed with H_2O_2. The expression of the ABA signal pathway genes *ABI1*, *RAB18*, *ERD15* and *ABF3*, and *SOS2* (SOS pathway) was altered in transgenic lines. These results suggest that *TaDi19A* plays a role in the plant's response to abiotic stress, and some possible mechanisms of its action are proposed.

Abstract reference: ITMI2009_143

A global effort in germplasm characterization and breeding for resistance to Russian wheat aphid in wheat and barley

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The Russian wheat aphid, Diuraphis noxia, is one of the most damaging insect pests in most wheat and barley growing areas around the world. This aphid is not yet present in Australia but its potential introduction would cause significant financial losses to the Australian grains industry. Because of this threat, a project has been initiated to allow extensive collaborations among RWA workers from a number of countries to facilitate germplasm exchange and provide excess to the RWA biotypes existing in different regions of the world.
The objectives of the study are to: 1) characterize available RWA resistant wheat and barley germplasm against available RWA biotypes around the world, 2) identify molecular markers closely linked to resistance genes, and 3) introgress RWA resistance into Australian wheat and barley backgrounds.
To date, 20 barley resistance sources and over 70 wheat lines were evaluated in standard seedling screening tests against a number of RWA biotypes collected from Mexico, Hungary, South Africa and France in Montpellier, France, and endemic biotypes in USA, Kenya, South Africa, Syria and Argentina. Lines with moderate to good levels of resistance were identified. Results from the testing of Australian barley lines against US biotype 1 in Oklahoma-USA, and wheat lines against a number of biotypes have shown that all were susceptible. Allelism tests, and diversity analysis of RWA resistant wheat and barley lines with the molecular markers will also be discussed. Validation of published markers and identification of new markers for new sources of resistances are progressing well. Introgression of resistance genes to Australian wheat and barley lines will also be reported.

Session 3:
COST WG3 Traitgen, Deciphering agronomical traits and phenotypes in the Triticeae

Abstract reference: ITMI2009_010

Cross Genome Map-based Cloning of a Nitrogen use Efficiency meta-QTL on Chromosome 3B in Bread Wheat Unravels New Evidence of Cereal Genome Evolution.

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In the last four decades, yield improvement in wheat has relied heavily on the widespread use of plant protection methods with high levels of fertilization. To cope with the challenges that agriculture faces today *i.e*. provide food, feed and bio-materials to a growing humankind while developing sustainable farming practices to limit its impact on ecosystems, the application of nitrogen fertilizers needs to be optimized. Therefore, it becomes of major importance to either select or produce new cereal cultivars with improved nitrogen absorption and metabolization while maintaining and even increasing yield. Even if Nitrogen Use Efficiency (NUE) is a highly heritable quantitative trait, the identification of the genetic basis and the cloning of QTL is very challenging in bread wheat that has a large (17 000 Mb) and unsequenced genome.

We performed a meta-QTL analysis for the identification of the most relevant genomic regions involved in NUE and found a major QTL on chromosome 3B. A genome-wide re-analysis of the synteny between cereals and wheat, allowed us to identify, with high confidence, orthologous regions corresponding to the wheat 3B NUE meta-QTLin rice, maize, sorghum and Brachypodium. The orthologous sequenced regions, were then used to automatically select 26 COS (Conserved Orthologous Set) markers for fine mapping on Advanced Backcrossed-QTL populations. Flanking COS markers were used to land onto the chromosome 3B physical map and select, sequence and annotate a 2.3 Mb region covering the meta-QTL confidence interval of 2.4 cM. Among the 10 annotated ORFs, a single gene has been structurally (association genetics) and functionally (correlation between gene expression and nitrogen status of the plant) validated. Sequence genome structure and evolution among grasses at the NUE locus will be discussed.

Abstract reference: ITMI2009_032

Functional diversification of barley FT-like genes in flowering

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FT, encoding one of PEBP proteins, promotes flowering and plays a central role in the integration of flowering signals from the vernalization and photoperiod in Arabidopsis. *FT* forms a small gene family in Arabidopsis with other 5 genes, whereas 19 and 25 PEBP genes including *FT* were identified in rice and maize genomes, respectively. This PEBP gene redundancy in cereals raises questions about the functional diversification. Arabidopsis is a long day (LD) plant, and its flowering is induced under LD conditions, whereas the rice *FT*-like gene *Hd3a* promotes flowering under short day (SD) conditions. These show that *FT* expression is common to flowering induction and that variation of *FT* expression is a key difference between LD and SD response. Barley is closely related to rice, but is a LD plant like Arabidopsis. Therefore it is interesting to compare the functions of PEBP genes between rice and barley. We analysed five barley PEBP genes to clarify their functions in flowering using transgenic, expression and QTL analyses. Introduction of *HvTFL1* and *HvMFT1* into rice did not result in any changes in flowering, suggesting that these genes have functions distinct from flowering. Overexpression of *HvFT1*, *HvFT2* and *HvFT3* in rice resulted in early heading, indicating that these genes can act as promoters of the floral transition. *HvFT1* transgenic rice showed the most robust flowering initiation. In barley *HvFT1* was expressed at the time of shoot meristem phase transition. These suggest that *HvFT1* is the key gene responsible for flowering in barley. *HvFT2* transgenic rices also showed robust flowering initiation, but *HvFT2* was expressed only under SD conditions, suggesting that its role is limited to specific photoperiodic conditions. Flowering activity in *HvFT3* transgenic rice was not as strong and was modulated by the photoperiod. These suggest that *HvFT3* functions in flowering, but that its effect is indirect. HvFT3 expression was observed in Morex, a cultivar with a dominant allele of *Ppd-H2*, a major QTL for flowering under SD conditions, although no expression was detected in Steptoe, a cultivar with *ppd-H2*. *HvFT3* was mapped to chromosome 1HL, which carries *Ppd-H2*. Sequence analyses revealed that Morex possesses an intact *HvFT3* gene, whereas most of this gene has been lost in Steptoe. These data strongly suggest that *HvFT3* may be identical to *Ppd-H2*.

Abstract reference: ITMI2009_088

Expression profiling of the Skp1-Cullin-F-box (SCF) E3 ligases during wheat grain development

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The wheat grain development process could be split into three distinct phases: cell division, cell filling and grain drying. The transition from state to another is relying on the fine regulation of expression of thousands of genes. During the last decade, the ubiquitin proteasome 26S (UPS) pathway has emerged as one of the most important processes involved in the regulation of gene expression through the specific degradation of regulatory proteins such as transcription factors. The ubiquitination reaction involves 3 enzymes denoted E1, E2 and E3. In the SCF (Skp-Cullin-F-box) E3 ligase complex, the subunit F-box confers the target specificity (Smalle and Vierstra, 2004). Recently, a study on rice development shows that 31 F-Box are differentially expressed during the grain development (Jain *et al*., 2007).

To study the expression of the SCF complex during the wheat grain development, we first analysed the homolog of the genes found by Jain and his colleagues. From the 31 F-box in rice, 26 F-box orthologs were found by bioinformatics analysis. Semi quantitative PCR analysis identified 2 F-box differentially expressed during the wheat seed development.

Moreover, we developed a microarray chip containing 416 genes potentially encoding SCF components. Expression analysis identified 15 genes differentially expressed during wheat grain development. The potential role of these genes will be discussed.

Smalle J, Vierstra RD (2004). The ubiquitin 26S proteasome proteolytic pathway. Annu. Rev. Plant Biol. 5:555-590.

Jain M, Nijhawan A, Arora R, Agarwal P, Ray S, Sharma P, Kapoor S, Tyagi AK, Khurana JP (2007). F-box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. Plant Physiol. 143:1467-1483

Abstract reference: ITMI2009_130

Genetic Dissection of a QTL for Grain Size in Wheat

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The yield of wheat is determined by the factors spike number per plant, grain number per spike and grain weight. Grain size also constitutes an important component of the domestication syndrome of crop plants. Since these traits are usually inherited in a quantitative fashion the use of the usual mapping populations, such as recombinant inbreds or doubled haploids only leads to the detection of QTLs, however, does not allow to trace the single genes. Therefore, the concept of advanced backcross breeding proposed by Tanksley and Nelson (1996) and the subsequent development of nearly isogenic lines (NILs) was applied to detect and further dissect a QTL for grain weight into a single Mendelian gene.

The previously described QTL for 1000-grain weight *QTgw.ipk-7D* associated with microsatellite marker *Xgwm1002-7D* was originally detected in a BC_2F_3 advanced backcross population of the German winter wheat variety 'Prinz' and the synthetic wheat line W-7984 (lab designation: M6) (Huang et al, 2003). We developed nearly-isogenic lines (NILs) carrying introgressions of M6 in the genetic background of 'Prinz' with varying size on chromosome 7D. The BC_4F_3 NILs had a 10% increased 1000-grain weight compared to the control group and the recurrent parent 'Prinz' and 84.7% of the phenotypic variance could be explained by the segregation of marker *Xgwm1002-7D*. The trait increased grain weight was strongly correlated with increased grain length and increased plant height, while the trait grain number per ear was stable between the NILs and the control group. By using homozygous recombinant lines it was possible to delimit the QTL *QTgw.ipk-7D* to an interval of approx. 1 cM flanked by the markers barc126, wmc405 and gwm44 on chromosome arm 7DS. We will continue to physically map the region by using a 7D chromosome-specific BAC library and to exploit the synteny to rice.

In general, our data support the concept of using nearly isogenic introgression lines for validating and dissecting QTLs into single Mendelian genes and open the gateway for map-based cloning of a grain-weight QTL in wheat.

Abstract reference: ITMI2009_168

Genomics of stress tolerance in low yielding environments

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Abiotic stresses such as extreme temperature, low water availability, high light intensity, high salt, and mineral deficiencies or toxicities can severely reduce crop plant productivity. In many cases, several types of abiotic stress challenge crop plants simultaneously. High temperatures, high irradiance, scarcity of water and nutrient deficiencies are commonly encountered under growing conditions but are frequently not amenable to management through traditional farm practices. Higher plants have evolved multiple, interconnected strategies that enable them to survive unpredictable environmental fluctuations. However, these strategies are not always well developed in the cereal cultivars grown by grain producers and most of the strategies are focused on plant survival at the expense of yield.

The genetic control of traits determining yield in water limited and low yielding environments are generally expected to be of low heritability, polygenic and many of the key loci will show epistatic rather than additive effects. Current breeding and mapping techniques make it very difficult to detect and select for these types of loci. Know confounding factors, such as maturity, height, resistance or tolerance to soil diseases, and tolerance to related stresses such as boron, acidity, salinity and nutrient deficiencies must be taken into account. In many cases the genetic control of tolerance to these factors is known so that they could be fixed in both breeding and mapping populations.

Many of the key traits influencing yield are poorly understood at the physiological level, hard to reliably phenotype and the genetic control is frequently poorly understood. However, whole genome approaches and systemic analysis of the molecular basis of stress tolerance responses are starting to reveal key pathways and process involved in maintaining yield in difficult environments. Results now coming out of genomics studies are providing new insights into stress responses and provide novel strategies to improve stress tolerance. A broad approach to using genomics techniques to tackle abiotic tress tolerance in wheat and barley will be presented with some specific examples of how these results can influence practical crop improvement.

Abstract reference: ITMI2009_129

Reproductive frost tolerance genes in barley

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Overnight frost events in the order of -5^{o}C during flowering can damage the tender reproductive tissues of wheat and barley, resulting in extensive sterility and yield loss, or damage to the young grains leading to quality downgrading. Despite several decades of efforts to breed for low temperature tolerance at the reproductive stage (LTR tolerance), locally adapted varieties with LTR tolerance are not yet available. LTR tolerance QTL on barley chromosomes 2H and 5H, with the tolerance deriving from Sapporo varieties Haruna Nijo and Amagi Nijo, represent the only cereal LTR tolerance loci identified to date. The aims of our work were to characterize and clone these tolerance genes, to help facilitate the use of these LTR tolerance genes in breeding, and to elucidate the mechanisms of LTR tolerance.

Temperature profiles of natural frost events were used to design protocols for using a frost simulation chamber. LTR tolerance encoded by each locus was detectable in the simulation chamber, and experiments using an ice nucleation spray indicated that these effects derived from freezing and not chilling. Several developmental traits have the potential to impact on LTR tolerance. However, based on genetic analyses in the two LTR QTL mapping crosses, none of these traits represent the basis for the LTR QTL. A locus (*Flt-2L*) controlling flowering time, plant height and spike density was identified in the vicinity of the 2H LTR locus, but we were able to show that this locus could be separated from the RFT locus by recombination. A close homologue of the wheat domestication locus *Q* was identified as a likely candidate for the *Flt-2L* gene. A novel flowering time effect was also found to be segregating in the vicinity of the 5H LTR tolerance locus in both LTR mapping crosses, and will require further investigation. A detailed picture of the rice-barley co-linearity across the LTR QTL regions was established by the generation of over 60 new PCR markers, providing a framework for the further delimitation and cloning of the LTR tolerance genes. Genetic strategies have been devised to allow the fine mapping of the subtle 2H and 5H LTR tolerance effects in the absence of interference from flowering time variation.

Abstract reference: ITMI2009_080

Allelic effects of some photoperiod and vernalization barley genes on flowering time under Mediterranean conditions

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Heading date is a trait of major importance, as one of the main determining factors of adaptation and yield in cereals. This is especially important in regions with limited water availability. The main factors controlling the time to flowering in cereals are the requirement of vernalization and the sensitivity to photoperiod and candidate genes for three vernalization and two photoperiod genes have been proposed. The objectives of this work were to assess the possible effects and type of actions of two vernalization response genes (*Vrn-H1* and *Vrn-H3*) and the photoperiod response gene *Ppd-H2* in two F_2 populations derived from winter crosses, Esterel x SBCC106 and Esterel x SBCC016, obtained in the framework of the Spanish national barley breeding programme. The first population segregates *Vrn-H1* and *Ppd-H2*. The second one segregates for *Vrn-H1, Vrn-H3* and *Ppd-H2.* The effects detected were dependent on population and time of sowing. No effect was evident in Esterel x SBCC106 sown in autumn. Effects were apparent in both populations only under winter sowing conditions. There were small differences between the two alleles of *HvBM5A* (*Vrn-H1*) for time to stem elongation and growth habit. *HvFT1* (*Vrn-H3*) was associated to large and significant effects on flowering time; plants homozygous for the SBCC016 allele (AG) were later than those plants carrying the allele from Esterel (TC). This gene showed additive as well as dominance type of gene action, and may play a role in the adaptation of barley to Mediterranean conditions. *HvFT3* (*Ppd-H2*) had a significant effect on flowering time in both F_2 populations (winter sowings). Plants null for this gene (recessive *ppd-H2* allele) flowered significantly later than those plants with dominant *Ppd-H2* allele.

Abstract reference: ITMI2009_135

Lower transcript levels of HvNIP2;1 at the Bot3 locus in barley confer increased tolerance to high boron supply

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Barley (*Hordeum vulgare* L.) is an annual cereal grain and has been ranked number four among the staple foods of the world. Today's cultivated barleys are not well adapted to high soil boron (B) although toxicity to B has been known for some time. The Algerian landrace Sahara 3771 proved of being highly tolerant to B and thus, represents one of the most B-tolerant barleys currently known. It carries four quantitative trait loci (QTL) conferring tolerance to toxic B conditions. One gene (HvBot1) underlying the tolerance QTL on chromosome 4H of barley has recently been identified and is a putative membrane-bound B transporter with similarity to bicarbonate transporters in animals; it functions as an efflux transporter to move B out of the plant (Sutton et al. 2007; *Science* **318:**1446 ff.). *Bot1* was the first B tolerance QTL to be identified in plants. In this work, we describe the identification of a second high B tolerance QTL in barley, mapping to the 6H B tolerance locus, *Bot3*. The predicted protein underlying *Bot3* belongs to the aquaporin superfamily of channels, and can be classified to the plant-specific aquaglyceroporins which are capable of transporting water, glycerol, and other small, uncharged molecules (Bhattacharjee et al. 2008; *J Biology* **7**:33). It can be further classified to the subfamily of nodulin-26-like intrinsic (NIP) proteins and hence, we named its gene product HvNIP2;1. Using heterologous expression systems in yeast and *Xenopus* oocytes, we will show that HvNIP2;1 can facilitate transport of B across membranes. Higher tolerance to B in Sahara 3771 is mediated through lower transcript levels of HvNIP2;1 in root tips of barley plants possibly owing to a repeat insertion into the promoter region of HvNIP2;1 approximately 2 kb upstream of the start codon. Moreover, we observed lower shoot B accumulation in a rice (*Oryza sativa* L.) mutant possessing a point mutation in the orthologous rice transporter gene (OsNIP2;1). Based upon our results we conclude that under high soil B *Bot3* entails lower shoot B accumulation and thus, effectually aids to higher B tolerance in Sahara 3771.

Abstract reference: ITMI2009_013

Molecular identification of the rye Pm8 resistance gene and its genetic suppressor in wheat

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The genetic improvement of tolerance to biotic and abiotic stresses is essential in wheat breeding. One successful strategy to improve yield and increase disease resistance has been based on the introgression of rye (*Secale cereale* L.) genes into wheat cultivars. The 1RS chromosome arm derived from the rye cultivar 'Petkus' carrying the race-specific resistance genes *Yr9*, *Lr26*, *Sr31* and *Pm8* is the most widely used rye-wheat translocation since it mediates resistance to yellow rust, leaf rust, stem rust and powdery mildew, respectively. So far more than thirty powdery mildew (*Pm*) resistance genes have been genetically characterized in wheat, but only an allelic series of the resistance gene *Pm3* has been cloned and molecularly analysed. We are aiming at the cloning and molecular characterisation of the rye powdery mildew resistance gene *Pm8* and its suppressor gene present in a subset of wheat lines. Identification of a resistance gene and its suppressor is of great interest considering the frequent occurrence of genetic suppression, especially in lines with introgressed alien chromatin. We have identified candidate genes for both *Pm8* and its suppressor. These are currently tested using a transient transformation assay.

Abstract reference: ITMI2009_106

Expression profiling of the wheat leaf rust Lr1 pathosystem using transgenic lines and the Affymetrix wheat chip

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Leaf rust resistance gene *Lr1* was cloned and 17 independent Fielder transgenic lines were generated to demonstrate gene complementation. In the incompatible interaction with *Puccinia triticina*, *Lr1* causes a hypersensitive response, observed on wheat leaves as flecks visible 10-14 days after inoculation. Unlike the majority of R-genes, *Lr1*'s mode of action is co-dominant, and, as such, plants heterozygous for *Lr1* exhibit an intermediate level of resistance. The *Lr1* transgenic lines are the perfect near isogenic material to decipher the genetic pathways of this gene-for-gene system. Two expression profiling experiments using the Affymetrix wheat chip were performed. A 27-chip experiment representing three genotypes (R-, H-, S-T_0-938 derived transgenic sister lines), three time points (0, 6, 24 HPI) and three replicates constituted the first experiment. A 21-chip experiment using 7 genotypes (6 *Lr1* transgenic lines and Fielder) at the same time points was also performed to provide greater assessment of the incompatible interaction. In the first experiment, 584 of the 61127 probe sets, representing 376 different transcripts or transcript families, were differentially regulated in at least one of the 6 possible genotype ´ time point interactions. The intermediate reaction shared 43 transcripts with the compatible interaction and 7 with the incompatible but the majority of the differentially regulated genes were unique to this interaction type at both 6 and 24 HPI. This reaction is therefore not simply the result of the simultaneous compatible and incompatible pathways but a more complex interaction between these pathways and over time. To confirm the differentially expressed genes in the incompatible interaction, we looked at 6 independent *Lr1* transgenic phenotypes. These biological replicates significantly increased the power of detection and indeed, a total of 3385 probe sets were found to be differentially regulated including 1308 at both 6 and 24 HPI indicative of the complexity of the gene networks involved in the early stages of the hypersensitive response. The differentially regulated genes were annotated and analyzed using Pathway Studio which resulted in a complex picture of gene networks involved in these responses to *P. triticina* infection. These pathways largely differed from the ones observed at later stages of infection of the race non-specific *Lr34* interaction. Mechanisms of action and key genes for the *Lr1* system were postulated.

Abstract reference: ITMI2009_105

Convergent evidence for genes underlying quantitative pahtogen resistance in barley

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Quantitative pathogen resistance is of high importance to plant breeders due to its durability. However, it usually is polygenic in nature and controlled by quantitative trait loci, which makes it difficult to handle in practice. Therefore, knowing the genes that underlie quantitative resistance would allow its exploitation in a more targeted manner. In order to identify genes that mediate quantitative resistance of barley to the powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*) we have combined a functional-genomics approach based on transcript profiling and transient-induced gene silencing (TIGS) with an association-genetic approach. Approximately 500 differentially regulated candidate genes of barley were silenced by RNAi followed by scoring of *Bgh* infection. Silencing of 50 of those candidates resulted in a significantly altered interaction phenotype of attacked epidermal cells with *Bgh*. These plus a number of candidate genes based on publicly available data were selected for re-sequencing in a panel of barley genotypes that differed in their quantitative resistance to *Bgh*. This forward-genetic approach revealed a number of genes that exhibited significant association with race-nonspecific seedling resistance. Two candidate genes mapped to chromosome 5H, within a QTL interval for seedling resistance to *Bgh*. Re-sequencing of 20 genes within 20 cM at this locus revealed low linkage disequilibrium and identified additional associated gene candidates including the cell-death regulator protein HvLsd1. In conclusion, the integration of functional-genomic with association-genetic approaches allow us to rapidly zoom into candidate-gene lists and genetic intervals of interest and hold the promise to accelerate the discovery of genes underlying complex, quantitative traits in crop plants.

Abstract reference: ITMI2009_108

A map-based cloning approach to unravel genes for basal resistance to biotrophic fungi in barley

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Genetic resistance is a highly effective way to protect crops against diseases. The early layers of plant defense, designated by the term basal resistance, can be completely effective resulting in non-host resistance to unadapted pathogens, or partially effective resulting in host quantitative resistance. Such resistance is of particular interest because it is highly durable. Genes for basal resistance mostly reside on "Quantitative Trait Loci (QTL)", each with a relatively small contribution to the resistance. We use the barley – cereal rusts and powdery mildews relationships as a model to study the genetic architecture of basal resistance.

An integrated genetic linkage map of barley with nearly 7,000 molecular markers was constructed and used as a platform to compare the genetic positions of QTL across seven mapping populations. We found a surprisingly large diversity in QTL for partial resistance with, for example, more than 100 QTL for cereal rusts having confidence intervals covering 77% of the integrated map. The integrated map also gave us the opportunity to genetically map genes of interest and to identify possible candidates to explain the resistance QTL. The incorporation of QTL into susceptible lines (near-isogenic lines, NIL) and the use of such NIL to develop populations of recombinants allowed determining the precise position of several of the QTL.

We constructed two bacterial artificial chromosome (BAC) libraries of barley cultivars containing the susceptible and the resistance alleles of our target QTL. The BAC libraries were used to construct a contig at one leaf rust resistance locus that led us to the identification of candidate genes to explain this QTL. The functional characterization of the candidate genes identified will provide the definite proof of their involvement in basal resistance of barley to such fungi.

Abstract reference: ITMI2009_173

Modifying protein and starch composition of wheat kernel for technological and nutritional improvement

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Quality improvement of durum and bread wheat have mostly focused on gluten proteins due to their important role in influencing pasta- and bread-making characteristics. The research activity of the past four decades has resulted in the production of a massive amount of information on this heterogeneous group of proteins. Integration of conventional and novel approaches along with the uses of proper genetic material have contributed to elucidate protein composition-functionality relationships, thus providing useful information to better address breeding programmes for quality improvement.

Though starch amounts to about 70% of the wheat grain its role in influencing quality characteristics of wheat end products has been less investigated. Identification of mutants for genes involved in the starch biosynthetic pathway has made feasible to manipulate starch composition; in particular, the ratio of the two major constituents, amylose and amylopectin, can be modified with the possibility to obtain durum and bread wheat cultivars with improved technological and nutritional characteristics.

Conventional and innovative approaches available quality improvement of durum and bread wheats will be presented.

Abstract reference: ITMI2009_027

Mapping Quality Traits Associated with Grain Micronutrient Content in Diploid Wheats Using Interspecific Introgression Lines

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Interspecific introgression lines (ILs) have been used extensively in plants as their power to detect QTLs with small effects is higher compared to mapping populations having whole genome fragments segregating. In addition, interspecific ILs are a valuable tool for adding foreign DNA in "elite" varieties to improve their agronomic traits. Sterility as well as low levels of viability can affect the development of intespecific ILs despite that they were successfully obtained for tomato, watermelon and barley.

Among *Triticum* species, two different A^u and A^m genomes derived from *Triticum urartu* and *Triticum monococcum*, respectively, were identified. A^u and A^m genomes share similar chromosomal organization; notwithstanding, significant molecular divergences have been assessed using sequence data and AFLP markers, supporting chimeric A^u/A^m chromosomes as a tool to dissect their genetic differences. The domesticated *T. monococcum* accessions L118 and the *T. urartu* accession ID388 differ in the grain content of Zn, Ca, carotenoids, tocols as well as in other important agronomic traits. We joined these hallmarks with IL properties to gain insights on the key loci controlling micronutrient content of kernel in diploid wheats.

Offsprings obtained from crosses between *T. urartu* and *T. monococcum* were reported to be sterile, but rare fertile F_1 plants were obtained by crossing the "elite" accession L118 with the wild accession ID388. From these fertile genotypes, ILs were obtained after several cycles of backcrossing using L118 as the recurrent parent. Afterwards, the ILs were fingerprinted using AFLP and previously mapped microsatellite markers, to characterize the introgressed fragment in each IL and to construct a panel of ILs carrying overlapping chromosome fragments of *T. urartu*. The phenotyping of the IL panel for some important traits associated with micronutrient content in kernel as well as statistical analyses searching contingent or transgressive QTLs are currently underway.

Abstract reference: ITMI2009_124

Multi-environment QTL mixed models for dissecting N use efficiency and tolerance to low N in wheat

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To achieve high yields, inorganic nitrogen (N) fertilisers are intensively applied on the wheat crop. These fertilisers represent a cost to the grower and have environmental impacts through N leaching, use of fossil fuels for their manufacture, and de-nitrification producing N2O that contributes to global warming. Farmers are also facing increasing economic pressures with N fertiliser currently at a very high price. Hence, there are both environmental and economic incentives to breed for higher N use efficiency.

The objective of this work were to identify chromosomal regions determining N use efficiency at different N levels. Two mapping populations were experimented in Northern France for two years in three sites. Two nitrogen supplies were tested at each location: a high N supply (N+) corresponding to the current agricultural practices and a low N supply (N-) where N applied was 60 to 144 $kg.ha^{-1}$ less than the high nitrogen supply. For both populations, fungicide, insecticide, and herbicide treatments were applied on both N levels to achieve an optimised management of the crop. Growth regulators were applied to prevent lodging. A genetic map mainly composed of about 200 micro-satellite markers was available for each population.

Grain yield, heading date, plant height, as well as straw yield, grain and straw nitrogen contents were measured on each plot. Soil mineral N was assessed in each location in February in the upper 90 cm or 120 cm of the soil profile.

The three major issues were: (1) an accurate estimation of single-trial performance (accounting for soil and nutrient treatment variability; (2) appropriate accommodation of the genetic correlations among environments (N treatments, sites and years) to account for genotype by environment interactions and (3) powerful but reliable methods to estimate QTL effects across and within different types of environments. For that purpose multi-environment QTL mixed models were used.

Data will be presented concerning the most significant regions determining N use efficiency and their interaction with the environment. This understanding and the genetic markers so developed will contribute to the development of new wheat genotypes that are able to use nitrogen more efficiently.

Abstract reference: ITMI2009_113

Molecular Dissection of Breadmaking Quality and Kernel Characters in Wheat

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Wheat is the world's second most important food grain crop and India is the second largest producer of wheat. Due to the rapid explosion of economic growth and urbanization in the country, there is a rise in standard of living making changes in dietary pattern and this has lead to increase in demand for convenience food items. Current trend of wheat research has been to improve the quality of wheat for the end use product. Considering the end products and end user of wheat, the important grain qualities, which need to be improved, are grain protein content (PC), grain size & shape, hectoliter weight (He), grain hardness (GH), mixograph and bread making quality (BMQ). Good BMQ is the major end use requirement for the baking industry. We have focused our efforts to identify and map the QTLs governing BMQ and grain size/ shape variation using two different mapping populations. In order to decipher BMQ into its components, a mapping population from HI977 X HD2329 was phenotyped at three different agroclimatic regions for three consecutive years, for Sedimentation volume (SV), Thousand-kernel weight (TKW), PC and GH as BMQ is influenced by all these traits. Linkage map of HI977 X HD2329 was of 3162 cM with more than 200 markers. For BMQ, Loaf volume (LV) which is a direct measurement, showed presence of 30 QTLs in 12 chromosomes with 5.85% to 44.69% phenotypic variation. Similarly, we identified several QTLs governing Sv, Gpc, Tgw, Tw and mixograph traits. For QTL analysis of grain size/ shape, TKW, Kernel length (KL) and Kernel width (KW) were analyzed from two location using Rye Sel 111 X Chinese Spring population. Significant QTLs for KL, KL and TKW were found on various chromosomes. Further, efforts are in progress to fine map the important QTL of LV and TKW.

Session 4:
Genome Structure and Evolution

Abstract reference: ITMI2009_118

Structure and Evolution of Wheat Centromeres and Pericentromeres

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We have previously sequenced and annotated 2 centromere-associated BAC clones from a *T. monococcum* (Liu et al. Chromosoma 2008) and showed the dominant role of centromeric retrotransposons (CRWs) in determining the structure and function of the centromere. We also showed that the CRWs were probably amplified during polyplodization. Sequence analysis indicated that all published sequences associated with wheat centromeres are different parts of an autonomous CRW. Finally, the Wgel retrotransposon was found to be much more abundant in the pericentromeric regions of the A genome compared to the B or D genome chromosomes, suggesting that this variation may contribute to the inhibition of homoeologous chromosome pairing in polyploid wheat. To study in more details the wheat centromere structure and function, we used the physical map of the hexaploid wheat chromosome 3B cv. Chinese Spring (Paux et al, Science 2008) to identify contigs containing CRW sequences. A contig of 1.1 Mb comprising 11 BAC clones has been selected for FISH and sequencing. FISH experiments clearly demonstrated the centromeric origin for the contig. The dominant role of the CRWs in determining the structure and function of wheat centromere was further investigated using the contig sequence. In contrast to other grass species, no long arrays of satellite repeats were found in the 3B centromeric region. FISH analysis revealed a stronger signal for BACs clones that carry a high amount of CRW sequences and are likely located closer to the centromere. BACs from the more pericentromeric regions comprised not only centromeric sequences, but also genome dispersed sequences. A retroelement enriched in the B-genome was identified and used to discriminate the A, B and D genome chromosomes. A conserved repeat, C743R1, was found in the LTR regions of typical non-autonomous *CRW*, while it was absent from autonomous CRWs suggesting that not all of the non-autonomous *CRWs* are derived from autonomous elements. Finally, we identified a retrotransposon enriched in the pericentromeric regions of D-genome chromosomes through the analysis of a BAC clone from *Ae. tauschii*. FISH and Southern analyses indicated that a large number of *CRWs* are conserved in the donor species of the three bread wheat genomes thereby possibly favouring amphiploid stabilization and fertility. This research also supports further the view that the pericentromeric region is critical in differentiation of genomes (Zhang et al. Genome 1996).

Abstract reference: ITMI2009_071

Sequencing, annotation and characterization of 17 Mb of chromosome 3B contigs provide novel insights into the wheat genome organization and evolution

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Our current knowledge of the wheat genome structure is limited to the analysis of individual BAC sequences and of random BAC end and plasmid sequences. To improve our understanding of the composition and evolution of this large and complex genome, we have sequenced and completely annotated 136 BAC clones assembled into 12 contigs of 0.5 to 3.1 Mb in size originating from different regions of chromosome 3B. This first set of large wheat contiguous sequences account for 17 Mb and contains 128 genes, 21 pseudogenes and 14 gene relics as well as 2963 transposable elements, representing 83.5% of the sequences. The average gene density is of 1 gene/114 kb with important local variations ranging from 0 to 10 genes per BAC. Gene distribution is not random with 77% of the genes being clustered into several small islands containing 3 genes in average and a slight gradient of gene density towards the telomeric regions. Interestingly, all Mb-sized contigs carry genes irrespective of their chromosomal location. Only 32% of the BACs are devoid of genes and the largest gene-free region is 709 kb indicating that gene islands are small and scattered all along the chromosome, thus supporting the idea that the whole genome needs to be sequenced to ensure access to the entire gene space. Only half of the predicted wheat genes are orthologous and syntenic with rice chromosome 1. The other half consists of genes present in rice but on different chromosomes. Non syntenic genes are found in almost all (9/10) sequenced gene-containing regions with large variations depending on their composition and chromosomal location. The highest level of rearrangement was observed for a disease resistance region with 2/3 of the genes at non orthologous positions whereas other regions exhibit >90% of syntenic genes. We also precisely annotated the 2963 transposable elements and identified 271 different families, including 155 new ones (at least 2 members), and 356 unknown single copy elements. TE composition shows large variations along the chromosomes with some families strictly found in centromeric or telomeric regions. Compared to isolate BAC sequencing, analyses of Mb-sized contigs give new insights into the genome structure by revealing, for instance, that most of TEs are complete and fragmented by nested insertions that can reach more than 200 kb.

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Abstract reference: ITMI2009_011

Structural analysis of subtelomeric chromosomal region of Triticum and Aegilops species

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Spelt1 and Spelt52 are families of highly repetitive subtelomeric tandem repeats of the *Ae. speltoides*, the most probable progenitor of the B and G genomes in polyploid wheats. Spelt1 and Spelt52 content has drastically decreased in *T. aestivum* relative *Ae. speltoides* during evolution. As a result, these sequences have not been identified on chromosomes of certain common wheat accessions by *in situ* hybridization, while stray short tandems tandem were detectable by blot-hybridization and PCR. Spelt1 is more widespread in polyploidy wheats with the BBAA and BBAADD genomes, in contrast, the presence of Spelt52 was amenable to identification by blot-hybridization only. The location of Spelt1 and Spelt52 at the chromosomal ends and low copy number in the *T. aestivum* genome are advantages enabling Spelt1 and Spelt52 to be used as probes for the choice of appropriate subtelomeric clones.

The BAC library from *Triticum aestivum* cv. Renan was screened using Spelt1 and Spelt52 as probes. Nine positive clones were isolated; of them, clone 2050O8 was localized mainly to the distal parts of wheat chromosomes by *in situ* hybridization. The distribution of the other clones indicated the presence of different types of repetitive sequences in BACs. Use of different approaches allowed us to prove that seven of the nine isolated clones belonged to the subtelomeric chromosomal regions. Clone 2050O8 was sequenced and its sequence of 119 737 bp was annotated. It is composed of 33% transposable elements (TEs), 10.9% Spelt52 (namely, the subfamily Spelt52.2) and five non-TE-related genes. DNA transposons are predominant, making up 24.6% of the entire BAC clone, whereas retroelements account for 8.4% of the clone length. The full-length CACTA transposon *Caspar* covers 11 666 bp, encoding a transposase and CTG-2 proteins, and this transposon accounts for 40% of the DNA transposons. The *in situ* hybridization data for the clones and their derived subclones in combination with the BLAST search against wheat mapped ESTs (expressed sequence tags) suggest that clone 2050O8 is located in the terminal bin, covering 5% of 4BL. Additionally, four of the predicted BAC_2050O8 genes showed significant homology to four putative orthologous rice genes in the distal part of rice chromosome 3S and confirm the synteny to wheat 4BL. Our work provides new insights into the microcollinearity in the terminal regions of wheat chromosomes 4BL and rice chromosome 3S.

Abstract reference: ITMI2009_046

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

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Gene duplication by polyploidy (homoeologues) or other means (paralogues) is a prominent feature of angiosperm evolution. We studied gene expression among three homoeologues of hexaploid wheat that evolved from a common progenitor about 3 million years ago (MYA) and came into a common nucleus at different times: ~0.5 and 0.01 MYA. Gene expression corresponding to each homoeologue was identified by sequence comparison of cultivar 'Chinese spring' ESTs and the results were confirmed by single stranded conformation polymorphism (SSCP) analysis of RNA using nulli-tetra lines. Of the 859 genes analyzed, 58% were expressed from all three homoeologues, 33% from two, and only 9% were expressed from one of the three homoeologues. The largest percentage of genes (14%) were expressed in anthers and the least (7%) were expressed in pistils. Whereas, the highest number of homoeologues/gene were expressed in roots (1.72 out of 3 homoeologues) and the lowest number were expressed from anthers (1.03 out of 3 homoeologues). In general, the proportion of expressed copies decreased with the increase in homoeologue copy number. The most significant observation was that homoeologues for 87% of the genes showed different expression patterns in different tissues and thus have likely evolved different gene expression controls. About 30% of the genes showed dosage dependence as the expression of homoeologues changed in response to changes in structural copy number.

Abstract reference: ITMI2009_033

Studies on genome organisation and evolution in the age of high throughput sequencing: So much data and so little time!

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The genomes of barley and wheat are large and repetitive and their gene pools very complex. Comparative analysis can contribute to our understanding of the structure of Triticeae genome and gene pools. In order to obtain a whole-genome sequence sample, we performed two runs of 454 (GS20) sequencing on genomic DNA of barley cv. Morex which yielded an approximate 1% of a haploid genome equivalent. Almost 60% of the sequences were comprised of known transposable element (TE) families while another 9% represented novel repetitive sequences. We identified almost 2,300 protein coding gene sequences and more than 660 putative conserved non-coding sequences. We found that several TE families are distributed unequally along chromosomes which was confirmed by *in situ* hybridisations of selected TEs. A comparison of the barley data with a large sample of publicly available wheat sequences showed that the TE composition of their genomes differs dramatically, despite their very similar genome size and their close phylogenetic relationship. To complement genome-wide comparative surveys with short 454 reads, we did detailed comparison of large genomic sequences from Triticeae to study intraspecies genomic diversity. There, we compared sequences at the *Lr34* locus of the wheat varieties *Chinese Spring*, *Renan*, *Glenlea* and diploid wheat *Aegilops tauschii*. Additionally, we compared the barley loci *Vrs1* and *Rym4* of the varieties *Morex*, *Cebada Capa* and *Haruna Nijo*. Molecular dating showed that the wheat D genome haplotypes diverged only a few thousand years ago while some barley and *Ae. tauschii* haplotypes diverged more than 500,000 years ago. In some sequences, we identfied breakpoints between ancient haplotypes, indicating that the Triticeae genomes are a heterogeneous and variable mosaic of haplotype fragments.

Abstract reference: ITMI2009_084

Dynamics and differential proliferation of transposable elements in the wheat genomes

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Transposable elements (TEs) constitute more than 80% of the wheat species genomes. We have compared sequences of several orthologous regions from different accessions of the diploid (*T. urartu:*AA, *Ae. tauschii:*DD and *Ae. speltoides;* SS), tetraploid (*T. turgidum:* AABB) and hexaploid (*T. aestivum: A*ABBDD) wheat species. Important variations in DNA size and composition were observed between species, ploidy levels and even accessions of a same species or ploidy level. The comparative study allowed determination of molecular basis of several DNA deletion events, and 97% of the deleted DNA was shown to be "TEs-mediated", through unequal crossing over, illegitimate DNA recombination or other unknown mechanisms. Dynamics and proliferation of transposable elements in the A and B genomes of the natural and relatively stable wheat allopolyploids *T. turgidum* and *T. aestivum*, as compared to that of their diploid relatives was analyzed. Analysis of TE sequence proportions, ratios of complete to truncated copies and insertion dates of class I retrotransposons showed that specific types of TEs (*Athila*, *Copia*, *Gypsy* and *CACTA* elements) have undergone waves of differential proliferation in the B and A genomes of wheat. As estimated from their insertion dates and confirmed by PCR-based tracing analysis, the majority of differential proliferation of TEs in B and A genomes of wheat (87% and 83% respectively), leading to sequence divergence, occurred prior to the allotetraploidization event that brought them together in *T. turgidum* and *T. aestivum,* less than 0.5 MYA. Our study suggests that this natural allotetraploidization, appears to have neither enhanced nor repressed transpositions. TEs proliferation as resulting from insertions, removals and/or combinations of both evolutionary forces, in relation to polyploidization events, will be discussed.

Abstract reference: ITMI2009_093

Sequence based comparison of Mega-base homoeologous regions of A and B genomes of Bread wheat

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Bread wheat (*Triticum aestivum* .L) is hexaploid and is expected to carry three homoeoloci per locus. The three sets of homoeoloci interact in a way as yet unknown to control the phenotypic expression at a specific locus. To tackle this most fundamental issue of polyploidy gene expression and crop improvement, we need DNA sequence information of homoeologous chromosomes. Using data from ongoing physical mapping projects in France (3B) and USA (3A) the current study examines the extent of homology between *the Fhb1-Rph7* region on chromosome 3B and its corresponding region on chromosome 3A by comparing putative coding and non-coding sequence to provide insight into organizational and sequence similarities between the homoeologous genomes. Nearly, 43 ESTs from *Fhb1* region on chromosome 3B were used to develop the EST-STS makers and identify the BACs and in turn contigs corresponding to *Fhb1-Rph7* region on chromosome 3AS. The order of the markers was highly conserved and 4 contigs spanning 28 BACs were identified and sequenced. The ~3Mb sequence was annotated and compared with homoeologous sequence on chromosome 3B. The results of this comparative sequence analysis will be presented.

Abstract reference: ITMI2009_153

Genetic diversity analysis of wild and landrace barleys using the BOPA1 Illumina genotyping platform

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Barley has been continuously cultivated in the Fertile Crescent for approximately 10 000 years in parallel with its wild progenitor *Hordeum vulgare ssp. spontaneum* which is still widespread across the region. We are interested in the genetic structures of wild and landrace barleys from the Fertile Crescent and the relationships with modern barley cultivars. We are studying populations of wild (268 accessions) and landrace (485 accessions) barleys with well described geographical information. These samples have been genotyped at 1536 SNP loci using the BOPA1 Illumina platform and results will be presented for genetic diversity/population genetics parameters, haplotype structure/sharing and geographical correlations. We will also present data showing that ascertainment bias in favour of cultivar genotypes in BOPA1 leads to under-estimation of landrace diversity, which can be largely nullified by selecting a 384 SNP subset with high diversity parameters in landrace germplasm. Furthermore, we show that 1536 SNPs give a modest gain over 384 SNPs for barley germplasm description which does not justify the increased cost; However, reduction to a set of 96 SNPs gives unacceptably low performance.

Session 5:
COST WG2A Bioinformatics, Data management and integration

Abstract reference: ITMI2009_182

Zippers, velcros and loose ends in comparative grass genomics: Haute Couture or Prêt-à-Porter?

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Abstract reference: ITMI2009_102

GrainGenes, the Triticeae Genome Database

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The GrainGenes database has been serving genomic and genetic data for the Triticeae since 1993. It includes genetic and physical maps, probes used for mapping, nucleotide sequences, bibliographic references and an addressbook of colleagues. The GrainGenes website includes additional information and publications, such as the Catalogue of Gene Symbols for Wheat, the Barley Genetics Newsletter and the Annual Wheat Newsletter. Recent additions to the database are the physical/genetic map of wheat chromosome 3B, the OPA barley consensus map, and the OPA/DArT map of the Oregon Wolfe Barley population. A new GrainGenes service is TAWG, the Triticeae Annotation Working Group, a public repository for annotated genomic sequences of wheat and barley. Soon GrainGenes will host The Hordeum Toolbox (THT), a database for genotyping and phenotyping data from the US Barley CAP project.

Abstract reference: ITMI2009_121

REPET: pipelines for the identification and annotation of transposable elements in genomic sequences

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Transposable elements (TEs) are mobile, repetitive DNA sequences almost ubiquitous among prokaryotic and eukaryotic genomes. Their impact on genome structure, function and evolution is now widely acknowledged. In plants, they represent a large amount of the genome, from 15-20% in *Arabidopsis thaliana* and 35-40% in *Oryza sativa*, to 70% in *Zea mays* and 80-90% in *Triticum aestivum*. With the continuous improvement of sequencing techniques, it is now possible to sequence large, highly repeated genomes such as the 17-Gb hexaploid wheat. To ensure accurate gene and regulatory elements annotation, automatic, scalable procedures to fully identify and annotate their TE content are much needed.

To this aim, we developed REPET, a framework displaying two parallelized pipelines, TEdenovo and TEannot. The first phase corresponds to a *de novo* approach*, i.e.* the definition of consensus sequences corresponding to TE families while the second phase is building a library with these consensus to mine the genome and detect TE copies.

The TEdenovo pipeline searches for repeats via a self-alignment of the input genomic sequences, then clusters the resulting high-scoring segment pairs (HSPs), and finally builds one consensus per cluster from multiple sequence alignments. At the most crucial step, the clustering of HSPs, several programs are combined to improve efficiency. When applied on *D. melanogaster*, 85% of the consensus are matching with already known TEs. Moreover, the *de novo* consensus corresponds to subfamilies rather than families. Hence, these structural variants reveal the complex, intricate evolution of different TE families inside the same genome.

The TEannot pipeline aligns a TE library with genomic sequences using several programs. The matches are statistically filtered and then combined. Simple short repeats are annotated along the way. A "long join" procedure is applied to connect TE fragments belonging to the same copy and thus reconstruct the insertion patterns (i.e. nested TEs). Finally, the annotation is delivered in common formats allowing their subsequent analysis in genome browser and annotation editor. REPET is used currently on 15 different genome projects (plants, animals and fungi) and its implementation in the wheat and barley annotation pipeline TriAnnot is underway.

Abstract reference: ITMI2009_134

Towards annotating Triticeae genomes by cross-species cDNA mapping

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The annotation of a genome is a crucial step of a genome sequencing project. Ab initio gene predictions tend to produce a large number of false positives, while full-length cDNAs (FLcDNAs) are useful for precise identification of gene structures. However, the numbers of available FLcDNAs are limited in Triticeae species and it is envisaged to utilize a wealth of cDNAs obtained from closely related species, such as rice and maize. Sequence differences between species generally hamper accurate identification of splice sites, and lead to artificial short introns. Therefore, to solve this problem, we developed an algorithm for correction of splice sites predicted by cross-species mapping. We applied our method to the rice and Arabidopsis genomes. Of 49,391 non-rice monocot FLcDNAs (wheat, barley, maize and sorghum), 35,164 were mapped on 20,991 loci of which 29.8% had not been identified with rice FLcDNAs. In addition, to evaluate the prediction accuracy, we compared the predicted gene structures with splice sites in the CDS regions determined by rice FLcDNAs. As a result, we found that 64% of the predicted loci had the same gene structures as those derived from rice FLcDNAs. The proportion of mapped FLcDNAs and nucleotide identity between species were positively correlated (correlation coefficient (R) = 0.76). The specificity values calculated for rice and Arabidopsis were also positively correlated with the nucleotide identity (R = 0.76). Hence, if we use FLcDNAs of less than 25% sequence differences in the CDSs, it is expected that more than half of them can be mapped with >50% specificity. We are currently working on cross-species mapping to eight plant genome sequences, and the data will be release from the RAP-DB.

Abstract reference: ITMI2009_171

Perspectives on using new high throughput sequencing technologies on decoding the genomes of plant species

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The use of new sequencing technologies on complex genomes of plant is just starting. The goal of long, continuous assemblies is still puzzling with these methods, because of the short lengths of individual reads, and also of the relative lack of efficiency of current assembly programs. We will discuss recent developments in these fields that can allow sequencing of these complex genomes with minimum requirements of Sanger technologies.

Abstract reference: ITMI2009_086

TriAnnot: an automatic pipeline for Triticeae genome annotation

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Wheat is the staple food for 35% of the world population and is the most widely grown crop worldwide. Challenge for the 21st century is to ensure wheat production in sufficient amounts and quality to meet the demand in an environmental friendly manner and in the context of climatic changes and a growing competition between the production of grain for food and/or biofules.

Today, plant genomics offer powerful tools to address these challenges. Indeed, recent International (IWGSC - *International Wheat Genome Sequencing Consortium*), European (ETGI - *European Triticeae Genome Initiative*) and French national (ANR) projects of sequencing and annotating each chromosome of an agriculturally relevant crop such as the wheat genome will allow the establishment of robust genomic resources.

Therefore, one of the major goals is to develop relevant bioinformatics tools to automatically annotate large amount of genomic sequences since wheat is 40 times larger than the rice genome. It is then necessary to develop and improve integrative annotation pipeline for BAC sequence analysis in the *Triticeae* to support the identification of candidate genes by structural annotation following the IWGSC annotation guidelines, as well as exhaustive annotation and classification of repetitive elements and other biological targets.

More precisely, the aim is to improve significantly the TriAnnot V1.5 already available on web (http://urgi.versailles.inra.fr/projects/TriAnnot/). The major tasks are: 1. To improve the RAP-like module developed in collaboration with NIAS (T. Itoh and *col.*, Japan) with the use of larger data banks and a more sophisticated graphical viewing with different levels of biological evidences of gene models; 2. To integrate REPET (Quesneville et al. 2005 PLoS Computational Biology 1:166-175) that is, by itself, a sophisticated pipeline for Transposable Element (TEs) identification and annotation; 3. To add new gene finders, especially Mgene (G. Rätsch and *col.*), and new modules such as PASA/EVM and protein domain analysis; 4. To develop a TriAnnot dedicated cluster (200 cpu) for intensive calculations.

The present available architecture and further improvements will be presented and, examples of BAC annotations and comparison with other pipelines (PIPE, MIPS; RiceGAAS, Japan) will be discussed. The need for organizing further the international wheat genome annotation community under the umbrella of the IWGSC will be discussed as well.

Abstract reference: ITMI2009_096

Wheat data on GnpIS, the INRA URGI information system

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URGI (Unité de Recherche Génomique-Info) is an INRA bioinformatics unit dedicated to plants and pest genomics. URGI develops GnpIS an information system dedicated to genomic and genetic data.
GnpIS, is a web based system composed of several applications (in Java and Perl) built above a relational database that includes integrated schemas for sequence data (GnpSeq), annotation data (GnpGenome), mapping data (GnpMap), expression data (GnpArray), proteomic data (GnpProt), SNP data (GnpSNP) and genetic resource data (SiReGal).
Data are submitted by the laboratories through an automatic Web submission tool which allows the checking and the data bulk loading. Web interfaces allow the biologists to query and visualize the data and navigate through them.
The ongoing developments are the creation of an interoperability between the genomic and genetic databases modules to allow integrated queries at genotype and phenotype level.
The two tools are a google-like search tool based on HibernateSearch technology and a BioMart (EBI) which allow complex queries between two or more databases.
GnpIS contains already a lot of wheat data with EST, genetic mapping, cytogenetic mapping, QTLs, physical mapping, SNP and genetic resources data.
This presentation will show these data in a demonstration of the GnpIS tools.

Session 6:
Molecular breeding

Abstract reference: ITMI2009_179

Wheat Molecular Breeding and Pre-breeding at CIMMYT

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Use of markers in wheat breeding and release of resultant varieties has progressed substantially from the cautious optimism of a decade ago. Routine screening in the CIMMYT wheat program is now applied to around 30 marker loci, with screening of around 40,000 lines per year generating around 70,000 data points. Moreover, MAS is now used routinely by partners in more advanced developing countries like China and India. Compare this with the early- mid 90's when it was so hard to find molecular polymorphisms in wheat that a wide cross (Opata x synthetic) was the focus of ITMI. For the more cynical breeders at that time it must have seemed unlikely anything useful would come from these efforts if such a wide cross was needed to find polymorphisms and develop a map.

For molecular technology to be useful in breeding it must better identify parents for crossing and select the best segregants from those crosses. At CIMMYT our molecular research builds on our comparative strengths; our germplasm base and phenotyping capability [both in-house and through the International Wheat Improvement Network (IWIN)]. These strengths allow us to actively collaborate with researchers in advanced research institutes, contributing to the science and translating it to application in wheat improvement for and by the developing world.

Examples discussed will include diversity analyses which have been applied to international nursery series. In combination with phenotypic and environmental data, this information can be used for association mapping, haplotype analysis and to direct Ecotilling efforts. These techniques have or will be applied to landraces, wild and alien wheat species to identify potentially useful new genetic variation and focus introgression and marker development on these 'best bets'.

Beyond identifying useful genetic variation, a lot of unglamorous work is usually needed to develop high throughput markers that are a cost-effective supplement or alternative to high-heritability phenotyping over the 2 selection cycles per year that we have for many traits. Other factors affecting the decision to use MAS include heritability of the trait and the proportion of variation that can be accounted for by known genes or regions, the importance of the trait relative to others undergoing selection and whether MAS can be applied for them. More detailed examination of these factors and findings from modeling some realistic scenarios will be presented.

Abstract reference: ITMI2009_015

Rhynchosporium secalis resistance in barley – from mapping to marker development and pre-breeding material

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Rhynchosporium secalis, the causal agent of scald, is still one of the most important foliar diseases of barley. So far, only three major resistace genes against this pathogen have been identified in cultivated barley (*Hordeum vulgare* ssp. *vulgare*). To integrate these resistances into breeding material is a very protracted process and to combine them in one breeding line is to date virtually impossible, as there are no diagnostic markers available for marker assisted selection.

Aim of this project is therefore to identify new resistance genes or new alleles of known loci, to develop diagnostic markers for these genes and to integrate these resistances into adapted breeding material. For this purpose five DH populations segregating for scald resistance were phenotyped in the greenhouse and then genotyped using SSRs and scald resistance specific STS markers. By this means the resistances of all mapping populations have by now been identified, including one gene, that was so far only found in wild barley (*Hordeum vulgare* ssp. *spontaneum*). Fine mapping of the regions containing the resistance loci is ongoing and will be followed by the development of diagnostic markers using several strategies such as AFLP poolscreening and chromosome walking via BAC libraries. Finally all developed markers will be validated using a extensive collection of resistance donors and resistant varieties and accessions of international origin and a set of *R. secalis* single spore isolates, that show distinct genetic distance and intensity of virulence, before being put into use for marker assisted selection in resistance breeding programms.

Abstract reference: ITMI2009_073

The validation and use of marker-assisted selection in NS wheat breeding program

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Most recent advances in modern biotechnology in general and molecular markers in particular, have led to the development of a number of novel approaches that offer the chance of making plant breeding more efficient and more cost effective. Today, a great number of important major genes and quantitative trait loci have been mapped with molecular markers and some of them prove to be very useful. However, reports on successful implementation of molecular markers targeting increased efficiency and reduced cost of wheat breeding programs are still quite limited. This paper provides an overview of the present process of integration of conventional and molecular breeding in the NS wheat breeding program.

Abstract reference: ITMI2009_120

Fine mapping and marker development for the crossability gene SKr on chromosome 5BS of hexaploid wheat (Triticum aestivum L.)

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Although wheat can be crossed with a wide range of related species, most adapted wheat varieties are incompatible because of the failure of pollen to fertilize the ovary, greatly restricting the germplasm that can be used for alien introgression. Inhibition to crossability is genetically controlled and a number of QTL have been identified to date. *SKr*, a strong QTL affecting crossability between wheat (*Triticum aestivum L.*) and rye (*Secale cereale* L.) was mapped at the distal end of chromosome 5BS using 50 recombinant SSD originating from a cross between the poorly crossable cultivar Courtot and the crossable line MP98. Two classes of crossability were distinguished with a 1:1 segregation as expected for a single major gene in a SSD population after 6 generations of selfing, thereby confirming the major dominant effect of SKr on crossability. High disruption of colinearity between wheat and rice in the telomeric region of 5BS did not allow to use rice as a new source of markers to saturate the SKr region in the first place. High density barley EST maps allowed us to circumvent the problem and served as an efficient bridge to reach sufficient resolution and finally establish a close relationship with rice chromosome 12. Wheat SSR markers were also derived from a BAC sequence carrying the Hardness locus that is located on the proximal end of SKr on chromosome 5BS. In total, 6 additional markers were mapped at the SKr locus and 400 kb of physical contig were established on both sides of the gene after screening the Chinese Spring BAC library with flanking markers. Sequence comparisons showed that the region spanning *SKr* in wheat corresponds to a 52 kb region carrying 6 genes in rice. New high resolution mapping populations are underway to achieve the map-based cloning of the SKr gene. Two SSR markers completely linked to the *SKr* gene were further used to evaluate a collection of crossable wheat progenies originating from crosses between non crossable French elite cultivars and the readily crossable lines VM202 or Courtot/FukuhoKumugi 5B, which crossability origin was unknown. A high association was observed between crossability and the alleles from the crossable lines at the SKr locus. This confirmed the major effect of SKr on crossability in wheat and provided very useful markers for the efficient introgression of *skr* alleles in elite varieties to increase genetic diversity in wheat and triticale breeding programs.

Abstract reference: ITMI2009_177

The future impact of genomics assisted approaches in maize breeding

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The US maize community has initiated a huge three-step project in 1998, including the sequencing of the maize genome, to obtain the complete sequence and structure of all maize genes and their locations on both the genetic and physical maps of maize. All the information generated is made available to the community, via the maizegenome.org website and from the EBI database in Europe. Now the maize genome is almost fully sequenced, which is great news for genomics, and so for maize breeding!
Having access to the genome contributes to crop improvement because :

- comparative genomic approaches make links using the gene information already available on model species, to understand and identify more easily the function of key genes and complex biological mechanisms involved in the agronomic traits of crops.
- The assembled genome sequence provides a good basis for developing a large number of markers (wet lab or *in silico*) in candidate genes or within gene rich regions that can be used in genetic studies (QTL and association mapping). Such tools facilitate and stimulate germplasm and allelic diversity characterization and increase breeding efficiency by marker assisted selection. Traits can then be bred directly (selection of the favourable alleles only).

Bioinformatics is an essential component of such studies because it connects data from very diverse origins (genetic, transcriptomic, phenotypic, mutants) to the genome sequences, to generate valuable information to be used in applied programmes.
All this, associated with the new high-throughput and low-cost technologies now available (in sequencing and genotyping) is already speeding up the identification of the most interesting genetic factors involved in agronomic traits, thus improving marker-assisted selection.
Breeders need to encourage all the initiatives that aim at sequencing our genomes of interest.

Abstract reference: ITMI2009_146

Application of molecular marker in cereals breeding

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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 the 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.

Knowing the location of these genes/traits and specific alleles offers the possibility to apply marker-assisted selection (MAS) in cereals, because one of the main objectives of plant breeding is the introgression of one or more favourable genes from a donor parent into the background of an elite variety. Marker-assisted selection allows plant selection at the juvenile stage from an early generation. For simply inherited traits, conventional PCR, which requires a small amount of DNA, has become very useful for screening large populations of segregating progenies. Unfavourable alleles can be eliminated or greatly reduced during the early generations through marker-assisted selection, focusing the selection in the field on reduced numbers of promising genotypes.

The complete restoration of pollen fertility with additional significant effects on reduction of infection by the ergot fungus (*Claviceps purpurea*) in breeding hybrid varieties in rye, virus resistance in barley and resistance to *Fusarium head blight* and *Septoria tritici* in wheat are leading targets in practical breeding. In all cases the breeding process can be accelerated by applying molecular markers for developing new material. The detailed results of such specific applications of molecular markers in rye, barley and wheat will be presented and discussed.

Abstract reference: ITMI2009_149

High throughput SNP discovery in wheat using methylation-sensitive digestion and next-generation sequencing

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An SNP discovery experiment is described, performed on two cultivars of common bread wheat (*T. aestivum*), consisting of digestion of genomic DNA with a methylation-sensitive restriction enzyme followed by nextgen sequencing using the Roche 454 FLX genome sequencer. More than 1.5Gb of raw reads per cultivar has been generated, using both the standard LR-70 protocol and the Titanium protocol. SNPs are detected ab initio by a dedicated bioinformatics pipeline, through fragment assembly and detection of high quality bases differing from their corresponding consensus. The pipeline distinguishes between intra- and inter-cultivar sequence variations occurring in the hexaploid genome and formats results in suitable form for downstream genotyping probe design. It is demonstrated that HpaII digestion as described enables to reproductively select corresponding portions of the genomes of two crop cultivars. Furthermore, the read length of the GS-FLX pyrosequencing method allows documentation of SNP sequence context, a requirement for downstream SNP genotyping experiments. Advances made by the new GS-FLX Titanium protocol are illustrated.

Technological advances

Abstract reference: ITMI2009_180

High throughput proteomics for SNP identification within breeding programs

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The key constraint to increased use of molecular markers in wheat breeding programs is the difficulty associated with the identification of relevant markers within elite breeding populations. NeoVentures Biotechnology Inc. has pioneered a different way of approaching this problem by developing a peptide analysis platform. The approach changes the focus from maximizing the efficiency of DNA sequence analysis within representative genotypes to maximizing the efficiency of the detection of sequence variation across breeding lines. With MALDI TOF analysis of protease treated target proteins it is possible to obtain sequence information from 96 genotypes simultaneously. NeoVentures has developed proprietary databases and analysis platforms to support the prediction of DNA sequence variation among genotypes by combining different protease treatments with knowledge of target gene sequences. This approach enables the prediction of novel SNPs without a requirement for primer design, cloning and sequencing. The concurrent analysis of all members of homoeologous gene families in a polyploid species such as wheat is a useful aid for the assignment of alleles to loci. NeoVentures is committed to working collaboratively with the global wheat community to increase understanding of allelic variation within known enzymes. Commercial terms for marker identification for wheat breeding programs can be obtained by contacting Dr. Gregory Penner (gpenner@neoventures.ca).

Poster presentations

Session 1:
COST WG2 PhysGen, Structural and functional analyses of the Triticeae genomes

Abstract reference: ITMI2009_025

Functional characterization of a wheat E3 ubiquitin ligase involved in the response to abiotic stresses

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The proteolysis based on protein ubiquitylation is emerging as an important regulatory mechanism of the signalling pathways involved in the plant responses to environment and hormone stimuli. The members of the huge family of the E3 ubiquitin ligases specifically recruit protein targets of ubiquitin conjugation. We isolated a *Triticum durum* gene (*6g2*) encoding a C3H2C3 RING-finger protein which acts as E3 ubiquitin ligase in an *in vitro* poli-ubiquitylation assay. We verified its predicted nuclear localization by localising the GFP portion of a 6g2-GFP fusion transiently expressed in onion epidermal cells. The gene is early induced during cold/light and drought stress and subjected to chloroplast-dependent intron retention upon cold stress. Moreover we demonstrated its involvement in the dehydration response by a transient induced gene silencing (TIGS) assay in barley, where dehydration sensitivity is evaluated from the DsRed fluorescence. To identify potential ubiquitylation targets of *6g2*, a cDNA library from cold/light treated leaves of wheat has been screened by two-hybrid system. The interactors are a MAP kinase and two transcription factors (VIP2 like and JUBEL1 like) involved in the regulation of plant development.

Abstract reference: ITMI2009_029

Towards the construction of a high density genetic linkage map of wheat chromosome 5A

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A high density genetic map is needed for anchoring BAC contigs during the construction of a physical map and for DNA sequence assembly. The International Wheat Genome Sequencing Consortium is dedicated to the development of physical maps of individual chromosomes as the first step towards the whole genome sequencing of hexaploid wheat. To undertake this challenge for wheat chromosome 5A, we rely on several mapping populations and different parallel approaches for marker development. Three mapping populations are being used: 1) a Chinese Spring x Renan (CSxR) F2 population; 2) an F2 population derived from CS x CS-*Triticum dicoccoides* disomic substitution line for chromosome 5A and, 3) a RIL (Recombinant Inbred Lines) population derived from Langdon (LDN) x LDN-*T. dicoccoides* disomic substitution line for chromosome 5A. For marker development, a Diversity Array Technology (DArT) platform specific for the short and long arms of wheat chromosome 5A has been established using DNA from flow sorted chromosomes, and includes more than 6000 wheat probes. So far, this array is under hybridization with one population (CS x R) while the other two populations are in the pipeline. Besides the DArTs, a set of SSR, RFLP-derived and EST-derived PCR-based markers, specific for 5AS and/or 5AL chromosome arms have been selected from databases and literature. After the assignment to chromosome 5A, performed using CS deletion and aneuploid lines, the markers are being tested for polymorphism between the parents of the three mapping populations. Polymorphic DNA fragments, specific for 5A, will be mapped in the available population(s). The resulting genetic linkage map of the wheat chromosome 5A will be presented.

Abstract reference: ITMI2009_031

The impact of 5B:7B translocation on molecular-genetic mapping of wheat chromosomes

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Translocation T5B:7B is the most frequent chromosomal rearrangement in common wheat. So far it was identified in more than 50 cultivars from different countries, and is especially common in France (nearly 40% of commercial varieties). Several wheat cultivars carrying T5B:7B, such as Apache, Arche, Autan, Aztec, Cezanne, were used in the development of mapping populations in France, which significantly complicate construction of genetic maps. This is due to the fact that polyvalent formation in meiosis of plants heterozygous for translocation results in distortion of chromosome pairing and segregation, which in turn causes a decreased recombination frequency between parental chromosomes, i.e., a diminished genetic variation. Secondly, genes or markers located at both sides of translocation breakpoints are tightly linked in one parent but segregated independently in another. This causes an incorrect assessment of distances between markers and erroneous linkage groups, which may have a large negative effect in QTL studies. The abovementioned problems however can be solved if the exact chromosome structure of individual lines constituting mapping population will be determined. Our study was aimed in karyotype analysis of 238 androgenethic dihaploid lines produced from the F1 hybrids of Arche (T5B:7B) x Recital (normal) cross. The results had shown that 85 ARE lines possessed normal 5B + 7B; one has normal 5B + 7BS+7BL telosomes, 145 lines possessed rearranged chromosomes T5B:7B; one had T5BS:7BS + tel 5BL, one carried chromosome T5BL:7BL + 1 or 2 telocentrics 7BS. In four lines translocated 5B:7B chromosomes were further modified as a result of secondary translocations, and secondary translocations (up to nine) involving other chromosomes were identified in 12 lines. These results suggest that segregation of normal and rearranged chromosomes in the progeny of wheat cultivars heterozygous for 5B:7B translocation is not random: (1) there was strong selection against genotypes with non-balanced chromosome complements and (2) there was an obvious tendency of preferential transmission of translocated 5B:7B chromosomes through the pollen. These data will be helpful for improving the molecular-genetic map of 5B and 7B chromosomes, for precise location of breakpoint position, for mapping genes known to control the development rhythm, and finally for developing a model describing segregation of markers located in close vicinity to translocation breakpoints.

Abstract reference: ITMI2009_044

Colinearity-based marker mining for high density mapping of the wheat Powdery mildew resistance locus QPm.tut-4A

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Infection of wheat by the fungus *Blumeria graminis* causes powdery mildew disease with serious yield losses. Recently, a non-race-specific powdery mildew resistance gene *QPm.tut-4A* was mapped to the distal end of the long arm of wheat chromosome 4A. The region spanning 10cM is flanked by DNA markers *gwm160* and *wmc232*. In an attempt to clone the gene, we are constructing a high density map of the region. To produce markers targeted to the *QPm.tut-4A* region, we studied colinearity of this region in wheat, rice and barley. The marker *gwm160* was mapped to the very last bin on deletion map of the wheat chromosome arm 4AL. All 91 single copy ESTs from the last bin of chromosome 4A were mapped on the rice genome. 48 of the ESTs had orthologous genes in rice and the largest group of 20 ESTs mapped to the distal end of rice chromosome arm 6S, suggesting that this is the colinear region in rice. The assumption was supported by mapping the nearest sequence-based marker *psr119* linked to the *wmc232* marker in our mapping population. The majority of the ESTs mapped to a 1.6 M region of rice chromosome 6, which contains 173 genes. The region showed colinearity with the distal parts of wheat chromosome arms 7AS and 7DS and barley chromosome arm 7HS, but not with the group 4 wheat homologous chromosomes. Using the colinearity data, 48 mapped ESTs from the distal part of chromosome arm 7HS were selected as potential markers of the *QPm.tut-4A* region. Our colinearity study between wheat, rice, and barley yielded 121 genes, which are being used to develop new markers to saturate genetic map of the *QPm.tut-4A* locus. This work has been supported by the Czech Science Foundation (award no. 521/08/1629).

Abstract reference: ITMI2009_050

Development-dependent changes in the tight DNA-protein complexes in barley

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The tightly bound to DNA proteins (TBP) is a protein group that remains attached to DNA with covalent or non-covalent bonds after its deproteinisation. Functional role of this group is not completely understood yet. The main goal of this study was to evaluate tissue specific changes in the TBP distribution in barley (*Hordeum vulgare*) genes in different phases of shoot and kernel development. To reach the goal we have investigated the TBP distribution along *Amy32b* and *Bmy1* genes encoding low pI α-amylase A and endosperm specific β-amylase correspondingly using oligonucleotide DNA arrays and characterized the polypeptide spectrum of TBP and proteins with affinity to TBP-associated DNA. In the *Amy32b* gene transition from watery ripe to the milky ripeness stage of kernel development was followed by decrease of TBP binding along the whole gene, especially in promoter region and intron II. Expression of the *Bmy1* gene coupled to ripening was followed by release of the exon III and intron III sequences from complexes with TBPs. The spectrum of TBPs appeared to be organ and developmental-stage specific. Development of the first leaf and root system (from Zadoks 07 to Zadoks 10 stage) was shown as followed by drastic increase of the TBP number in contrast to coleoptile, where TBPs spectrum became poor during senescence. It was demonstrated that a nuclear protein of low molecular weight similar to described TBPs possessed high affinity to the DNA involved in TBP-DNA complexes.

Abstract reference: ITMI2009_054

Another Brick in the Wall: Building a Complete Set of Chromosome-Specific BAC Resources for Hexaploid Wheat

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The huge size of 17,000 Mbp, presence of three homoeologous genomes A, B and D, and prevalence of repetitive DNA make genome analysis in wheat an intimidating task. A solution to reduce genome complexity was provided by a possibility to isolate particular chromosomes using flow cytometry and clone chromosomal DNA into BAC vector. This technology offers a broad range of applications such as targeted development of markers from BAC end sequences, positional cloning and construction of physical maps. DNA pools can be prepared from clones of minimal tiling path for particular chromosomes and used to sequence wheat genome by the next-generation sequencing technologies 'per partes'. Early projects led to the construction of the first-generation chromosome-specific BAC libraries from chromosome 3B and chromosome arms 1BS, 1RS and 3AS. The mean insert size in these libraries was relatively small (typically 75-85 kb) due to a limited amount of chromosomal DNA, which permitted only one size-selection step. Currently, the second-generation chromosome-specific BAC resources in wheat are being produced. The improved protocol, which made the second size-selection step feasible, was used to construct BAC libraries from chromosome arms 1AS, 1AL, 3AL, 3DS, 3DL, 7DS, 7DL, and from pooled chromosomes 1D, 4D and 6D, with the average insert size exceeding 100 kb. Moreover, the increased BAC cloning efficiency enables construction of libraries from a smaller amount of chromosomes under less stringent size selection. Such libraries can be constructed for positional cloning of genes that are not present in cv. Chinese Spring, which is used for the development of a physical framework map of hexaploid wheat. Recent improvement of the protocol involves the use of MegaX DH10B competent cells (Invitrogen), which confer resistance to lytic bacteriophages T1 and T5. Two BAC libraries have already been constructed using these cells from chromosome arms 1BS and 1BL. This work has been supported by the Czech Science Foundation (award no. 521/07/1573), the Czech Republic Ministry of Education Youth and Sports (award no. LC06004) and the European Seventh Framework Project FP7-212019 'TriticeaeGenome'.

Abstract reference: ITMI2009_059

Transriptomic analysis of drought and heat responses in durum wheat

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The productivity of wheat, one of the most important crops worldwide, is often limited by an array of abiotic stresses that avoid a successful growth and a complete grain filling. The water shortage is the major limiting factor affecting crop production. Nevertheless drought stress never comes alone. Sometimes drought stress is associated to freezing temperatures that reduce water availability, sometimes to pathogens involved in vascular mobility, or to high soil salinity. But dramatic effects occur when high temperatures and drought stress happen simultaneously.
Recently several researchers endeavoured to dissect the complex responses to drought and heat stress in bread and durum wheat. Anyway the above works are far from field condition, where, in summer period, the two stresses occur at the same time.
In this experiment two different durum wheat cultivar were analyzed. Ofanto cv. was achieved from recent breeding programs and is characterized by high yield and good stability of production in stress conditions (drought and high temperature). On the contrary Cappelli cv. is one of the first italian cultivars and was developed in 1915. As consequence it is a low performance cultivar mainly in stressed conditions. An Ofanto x Cappelli molecular map and several QTLs for abiotic stress tolerances are also available.
These genetic materials were grown in drought stress condition, subjected to high temperature and to both stresses. A transcriptome analysis was carried out using the Affymetrix 61K wheat chip on three biological replicates of mRNA extracted from flag leaves during booting stage, a phase more susceptible to drought and heat stress.
The poster will present data on genes and pathways up- or down–regulated highlighting the common and the different molecular mechanisms actived by the two cultivars. Moreover a clustering organization of the genes will show specific drought and heat responses, with particular regard to specific genes activated only in the combined stress.
Final purpose of this work is to associate specific QTLs to the expression values of drought and heat tolerance candidate genes.

Abstract reference: ITMI2009_063

Whole genome physical mapping in barley

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The development of a physical map for the barley genome (*Hordeum vulgare* cv. Morex) is based on the high information content fingerprinting (HICF) of 550,000 BAC clones. BAC clones were derived from 5 different BAC libraries (2x *Hin*dIII, *Eco*RI, *Mbo*I, random sheared). To have an overview of the quality of all the libraries we evaluated them with quality parameters like neighboring- and chloroplast-contamination, comparison of number of fragments (after HICF) to insert size and empty vector or wells. All valuable fingerprints after analysis will enter the *de novo* contig assembly with FPC V9.0 software.
In January 2009 a contig assembly with 373,574 BACs (8.6 x genome coverage) was performed. Since HICF analysis is still ongoing and anchoring of BAC contigs has started only recently the contig assembly is performed at high stringency parameters only which consider only strong overlapping of BACs. The latest assembly resulted in 28,470 contigs with 84% BACs in contigs. Until August 2009 we prepare for a new assembly with approx. 490,000 clones and results will be presented.
In addition the anchoring of BAC-contigs to existing genetic maps is in progress. The first results of anchored contigs will be shown.

Abstract reference: ITMI2009_064

From plants to genes: construction of plant BAC libraries linked to high-throughput screening pipeline

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The French Plant Genomic Resource Centre (CNRGV) created in France in 2004 by the INRA (French National Institute for Agricultural Research), is a non-for-profit service centre dedicated to plant genomics. The objectives of the CNRGV are to gather, to conserve and to manage genomic libraries but also to provide high throughput molecular tools to the scientific community. The CNRGV is already in charge of more than 6 millions samples among more than 50 plant genomic libraries of model and crop plant.

The genomic libraries mostly represented by BAC and cDNA 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 like wheat.

The construction of BAC libraries has allowed the cloning of several genes through map-based cloning. However, positional cloning remains slow and tedious since genomic resources are rarely adapted for efficient, high throughput and specific screening. Screening of libraries is mainly made by hybridization on high density filters, which suppose to work on large number of membranes with hazardous radioactive products handling.

To resolve high density filters limitations, the CNRGV has developed a rapid and efficient tool to isolate positive clones located in regions of interest. Three-dimensional DNA pools (column-pools, raw-pools, plate pools and Super pool) have been created for wheat and tomato BAC libraries. DNA was produced then from each pool by the rolling circle amplification method with random primers and the phi29 DNA polymerase. Our efficient 3D-pools production methods combine the screening by real-time PCR which ensures high throughput, great sensitivity, and better traceability using SSR, EST, BES markers.

PCR screening of pools can be widely used for isolation of genes of interest, construction of physical map of a region, comparison of genomic studies between crop and model species. We will describe the use of this efficient pipeline for the screening of BAC libraries for important projects.

These tools are available for the scientific community, for genome analysis, physical mapping, map-based cloning and sequencing projects (http://cnrgv.toulouse.inra.fr/).

Abstract reference: ITMI2009_066

Genetic anchoring of the physical map of barley (Hordeum vulgare L.)

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The construction of a physical map of the barley genome is underway based on the approach of High-Information-Content-Fingerprinting (HICF) (Schulte et al. 2009, Plant Physiology 149: 142-147). Before such a physical map will become fully effective, integration of BAC contigs with a high resolution, high density genetic map is required. Genetic anchoring of the barley physical map is approached currently in three different ways: (i) PCR screening of multidimensional BAC DNA pools, (ii) array-based screening of multidimensional BAC DNA pools, (iii) hybridization of high density colony membranes with gene specific markers. Multidimensional DNA pools were provided for a BAC library (*HVVMRXALLeA*) representing 3.7 haploid genome equivalents. The first round of screening is performed on the 55 Super pools each containing 2688 clones. Subsequently the second round of PCRs are performed on Matrix pools for a specific Superpool identified find in the first step. The pooling strategy has reduced the total number of PCR reactions required to identify a precise BAC address to twenty six. As of August 2009 over 1600 EST associated SNP markers were screened on superpoools and the matrix pool screening is in progress. The feasibility of array-based multiplex screening of DArT markers (hypomethylated restriction fragments sampled across the genome) was evaluated, employing a set of low complexity multidimensional BAC DNA pools from the same library. Preliminary results demonstrated that this approach can be used efficiently to generate anchor points in a high-throughput manner. Screening of the BAC libraries by hybridization to high-density colony arrays is the third approach followed for anchoring of the physical map. High density colony filters are available for several libraries used for HICF analysis and screening by hybridization will be employed specifically to asses the extent and nature of locally duplicated genes in the barley genome.

Abstract reference: ITMI2009_067

Insertion Site-Based Polymorphism: A Swiss army knife for wheat genomics

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In wheat, transposable elements (TEs) account for more than 70% of the genome. They are the most significant factors in determining the genome structure, as well as key drivers of the wheat genome evolution. Therefore, TE-based molecular markers represent ideal tools for saturating the wheat genetic maps and for studying the structure and evolution of the hexaploid wheat genome. We have recently demonstrated the potential of using BAC-end sequences from specific wheat chromosomes for developing Insertion Site-Based Polymorphism (ISBP) markers that are based on the PCR amplification of a sequence spanning a junction between a TE and its flanking sequence using primers designed on both sides of the boundary. Since TEs are ubiquitous, ISBP markers have no distribution bias along wheat chromosomes. And with an estimated density of one marker per 5 kb, they represent an almost infinite source of polymorphism in wheat. Moreover, they are adapted to a wide range of detection techniques and show a high level of inter- and intraspecific polymorphism even in the elite wheat pool. These features make ISBPs very powerful tools for genomics analyses in wheat.

ISBPs were tested on European and Australian elite lines to assess their usefulness for marker-assisted selection. Results showed that these markers meet the 5 main challenges of MAS (reliability, DNA quantity and quality, technical procedures, polymorphism and cost), and consequently represent useful tools for wheat breeders.

Evolutionary studies were also performed by genotyping diploid, tetraploid and hexaploid wheat accessions, as well as wild emmer wheat (*Triticum dicoccoides*) from 17 populations representing regional patterns as well as contrasting microsites in Israel. These studies provided new insights into the wheat B-genome evolution and revealed correlations between gene diversity and environmental factors such as soil type, altitude and sun exposure (sun *vs*. shade), strongly suggesting an impact of these factors on TE transposition and subsequent TE-induced genomic variability.

Altogether, our results demonstrate that ISBP markers are very powerful tools for wheat genetics and genomics and that they that are likely to allow saturating genetic maps and subsequently unlock many doors leading to efficient genetic diversity studies, recombination and linkage disequilibrium analyses, association mapping, fine mapping and cloning of QTLs, as well as marker assisted-selection.

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The LTR Retrotransposons of Brachypodium distachyon

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The Triticeae tribe of grasses, including wheat, barley, and rye, are part of the Subfamily Pooideae. The subfamily includes as well the agronomically important *Lolium* and *Festuca* of the Tribe Poeae and *Avena* of the Tribe Aveneae. Although rapid progress is being made in accessing the genomes of wheat and barley, the Pooideae as a whole have until now lacked a reference genome sequence. However, the genome of the wild grass *Brachypodium distachyon*, which is a member of the Pooideae, has now been sequenced. Most grasses have large genomes, the Class I LTR retrotransposons constituting the majority of the genome space. However, the *B. distachyon* genome comprises only 270 Mb. The degree of retrotransposon abundance in genomes results from the relative rates of gain and loss of these elements. The LTR retrotransposons propagate by a "copy and paste" mechanism, which leaves the mother copy intact but produces cDNA copies by reverse transcription that are then integrated into a new genomic site. Once integrated, retrotransposons or parts thereof may be lost by various recombinational processes. We have therefore investigated the LTR retrotransposon component of the *B. distachyon* genome, with an eye to understanding the dynamics of replication and loss of these elements. The relative success of the *Copia* and *Gypsy* superfamilies, as well as the interaction between autonomous retrotransposons, which encode their own replicative machinery, and the parasitic non-autonomous elements, are also under study.

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Development of DArT markers from isolated chromosomes/arms to saturate genetic linkage map of hexaploid wheat

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High-density genetic linkage maps facilitate isolation of markers tightly linked to traits of interest, map-based gene cloning as well as anchoring BAC contigs and shotgun sequences. However, the cost of development and screening thousands of markers, which are needed to achieve required marker density, may be prohibitive. One of the marker systems offering solution to this problem is Diversity Array Technology (DArT). As the DArT markers are typed in parallel, it is possible to identify larger numbers of polymorphic markers in a single experiment with the cost per marker assay in many species around or even below US $ 0.01. Here we describe how the DArT can be coupled with chromosome flow sorting to target specific genome regions of hexaploid wheat, to significantly increase marker density and to construct high-resolution genetic linkage maps. Chromosome fractions containing all D-genome chromosomes, chromosome 3B and the short arm of chromosome 1B (1BS), respectively, were isolated by flow cytometric sorting and their DNA was used to develop genotyping arrays (>15,000, 384 and 2,688 clones were derived from D-enriched, 1BS and 3B fractions, respectively). We then performed mapping on arrays containing markers from chromosome-specific libraries: 59/68 of the 1BS-derived markers (87%) mapped to chromosome 1B while 567/711 of the 3B-derived DArT markers (80%) mapped to chromosome 3B. We were also able to compare the number of markers mapping to a specific chromosome in the current chromosome assignment of 4,448 DArT markers against their source (sorted-chromosome library versus random libraries). While only 2,688 clones (probes) were derived from 3B compared to over 70,000 clones from the whole genome, 549 of 846 DArT markers (65%) assigned to chromosome 3B are from the '3B-sorted' library. The number of markers obtained in this study significantly exceeds the number of currently available markers for the selected wheat genome regions and confirms the advantage of our strategy. Only about 5 ng chromosomal DNA, which can be obtained from 5-15,000 chromosomes/arms, is needed to develop a DArT array. As this amount of chromosomes can be obtained within 1-2 hours of sorting, the new approach is generally applicable to all crops for which methods for chromosome sorting are available. This work has been supported by the Czech Science Foundation (award no. 521/07/1573) and the Czech Republic Ministry of Education Youth and Sports (award no. LC06004).

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Isolation from a wheat genomic BAC library of clones containing the sequence of Dehydration Responsive Factor (DRF1) gene for revealing its regulatory regions and possible physically closely related genes.

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Drought, enhanced by the global climate changes, is one of the most severe abiotic stresses limiting crop productivity. The importance of crop resistance to water stress is further increasing, as the world population continues to expand, and significant advances in understanding the molecular mechanisms of the stress response are required.
The *Triticum durum* Dehydration Responsive Factor 1 gene, *TdDRF1*, a *DREB2*-like gene, is expressed in response to dehydration and encodes for key stress-responsive transcription factors imparting stress endurance to the plants. Recently, the expression profile of this gene was finely examined in several wheat varieties and all obtained data spotlighted that the targeting of this gene could help the selection of cultivars for improving plant tolerance to drought.
The current research aims at getting a fine mapping of this gene, determining the exact locus position on chromosomes. At the moment, it is already established that homeologous loci of this gene exist in 1A, 1B and 1D chromosomes of bread wheat and in 1A and 1B chromosomes of durum wheat.
A bread wheat (cv. Chinese Spring) genomic BAC library was deeply investigated for getting specific clones containing the sequence of *TdDRF1* gene, by both superpools/pools PCR screening and macroarrays hybridization. A durum wheat (cv. LDN#65) genomic BAC library is still under screening.
Several BAC clones containing the target sequence were identified, PCR validated and characterized by restriction analyses for attempting the reconstruction of their "linear consequentiality" along the chromosome. Moreover, upstream and downstream gene regions were sequenced by chromosome walking and analysed to infer some information on promoter and other regulatory elements.
Furthermore, the complete sequence of some selected BACs (average BAC insert size ~ 130 Kb) is going to reveal if some other dehydration-responsive gene is located close to the target locus and this information will be precious for developing applications in molecular assisted breeding/selection programs aimed to the release of drought tolerant wheat cultivars. Preliminary sequence results will be discussed.

Abstract reference: ITMI2009_083

TILLING for low phytic acid (lpa) seed mutants in wheat

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Phytic acid (PA) is the main storage form of phosphorus in cereal seeds. Phytic acid is recognized as an anti-nutritional factor as neither humans nor swine and poultry are able to digest and utilize PA. Since PA is a strong chelator of important minerals such as Fe, Zn, Mn and Ca, this may contribute to lack of micronutrients in human populations where cereals are the primary source of nutrition. Furthermore, in husbandry excretion of PA can lead to accumulation of P in soil and water, and subsequently to eutrophication. One way to deal with the problems outlined above would be to identify low phytic acid (lpa) mutants impaired in PA biosynthesis or transport. Since a number of genes affecting these processes have been identified in cereals, particularly in rice, barley and maize, one strategy to identify a large number of lpa mutants would be to use TILLING (Targeting Induced Local Lessions IN Genomes).

In our group we have developed six TILLING populations of spring wheat, including one (Amaretto) where two rounds of EMS treatment have been conducted to increase mutation frequency. This population (M_2) is currently screened for mutations using primer pairs specific for each of the three homologue wheat genes encoding *myo*-inositol 3-phosphate synthase (MIPS), which catalyzes the first committed step in the biosynthesis of PA. The mutation frequency in the Amaretto population is rather high as an average of 2-4 mutations are detected each time 100 plants are tested with one of the MIPS specific primer pairs. Further screenings will be conducted to confirm this mutation frequency. Mutations causing amino acid changes have been identified in the three wheat MIPS homologues and these are currently tested for high content of free phosphate in the grains to identify mutations with an effect on the phenotype. In addition, the three wheat MIPS genes have been physically mapped to wheat chromosomes using a set of wheat deletion lines.

Abstract reference: ITMI2009_109

Saturation of genetic linkage map in durum wheat and QTLs for yield and yield stability

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We have analyzed two RILs populations from intraspecific crosses; the first of these used in recent past to construction the first molecular linkage map of durum wheat. The RILs have been produced, respectively, from cultivars ChamI by Jenna Ketifa (113 genotypes) and ChamI by Lahan (112 genotypes). These cultivars are developed at International Center for Agricultural Research in Dry Areas (ICARDA). Parents of these populations where chosen since they were polymorphic for a wide number of markers. Cham I cultivar present wide adaptation with high yield potential and yield stability; is early, resistant to yellow rust and aphid, but is susceptible to leaf rust and powdery mildew. Additionally, it shows high values of osmotic adjustment and quality. Jenna Ketifa cultivar is a landrace collected in 1990 in the south east high plateau of the Atlas Mountains of Morocco, it shows specific adaptation to North African continental dryland; it is tall, moderately resistant to drought, could, leaf rust, powdery mildew and to septoria, but is susceptible to yellow rust. Lahan cultivar is widespread diffuses in most wheat growing areas; it was appreciated for its high yield, good grain size and frost tolerance.
The analyses results in a genetic linkage map with a higher number of markers to increase the saturation of the published one and a new intraspecific genetic linkage map. Moreover, the RILs have been analysed for agro-morphological traits in several locations from North Africa and West Asia, so QTLs for yield and yield stability are reported.

Abstract reference: ITMI2009_112

Sequencing of bulked BAC clones on chromosome 3H of barley physical and genetic maps

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Haruna Nijo BAC library was applied for clone selection by primer sets derived from genetically and physically mapped ESTs on chromosome 3H and the pooled barley BAC clones were sequenced by 454 parallel genome sequencer. For the selection of genetically mapped ESTs, a high-resolution transcript linkage map of barley was created using a single doubled haploid mapping population, only 3'-end ESTs, and only PCR-based assays. Cultivar "Haruna Nijo' and an ancestral wild form accession "H602" were used as EST donors and crossing parents of the mapping population. Of the 2,890 ESTs mapped by SNPs (1,717), CAPS (933) and INDELs (240), 444 ESTs were mapped on chromosome 3H. For the selection of physically mapped ESTs, we used the gametocidal system inducing chromosomal structural changes in the 3H addition line of common wheat, and we cytologically screened for rearranged chromosomes involving the 3H chromosome by in situ hybridization (FISH/GISH). We used these dissection lines to map 36 EST markers that were polymorphic between euploid common wheat and the 3H addition line and that had been used for the construction of a 3H genetic map. The results of the PCR analyses placed the 36 EST markers into 20 chromosomal regions flanked by the breakpoints of the dissected chromosomes. These markers were used for BAC clone selection. The DNA samples of 10 or 20 BAC clones were pooled and used for shotgun library development. Contig sequences generated in each pooled library were compared homology with mapped EST sequences. Homology and colinearity between genome sequence of rice chromosome 1 and contig sequences from barley chromosome 3, which are arranged by genetic and physical distances, are discussed.

Abstract reference: ITMI2009_117

TriticeaeGenome Project

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For many years the size and complexity of the wheat and barley genomes have hampered the development of genomics and its application to produce Triticeae crops with improved composition and characteristics. Recently, however, new and more efficient scientific capabilities and resources have been developed that allows robust genomic programs to be established for the Triticeae. The TriticeaeGenome European FP7 project (Genomics for Triticeae improvement) is designed to achieve significant progress in Triticeae genomics and to support efficient breeding of improved varieties for the European agriculture by:
(i) Constructing anchored physical maps of wheat and barley chromosomes of group 1 and 3, which carry a large number of important agronomic traits;
(ii) Isolating 5 genes and QTLs (Quantitative Trait Loci) underlying disease resistance, yield and, and quality traits in wheat and barley;
(iii) Identifying and exploiting new alleles for the isolated genes through the use of mutant populations and genetic resources;
(iv) Supporting the development through molecular breeding of new varieties that meet farmer, processor, and consumer needs;
(v) Developing new bioinformatics tools to integrate and display large-scale genomics data;
(vi) Coordinating and integrating TriticeaeGenome research through interactions with other projects at National, European and International levels, for enhanced efficiency and mutual benefit
(vii)Providing training in emerging technologies, disseminate the results and transfer know-how to industry and users.
TriticeaeGenome is being implemented as a key contribution to efforts by international consortia to construct physical maps of barley and hexaploid wheat for improving plant breeding, accelerating gene and QTL isolation and setting up the foundation for future genome sequencing. It will deliver novel information and tools to breeders and scientists for a better understanding of Triticeae genome organization, evolution, and function and thereby provide a better understanding of the biology of these essential crops, enabling significant improvement of their composition and characteristics to satisfy the needs of European consumers, processors and producers. TriticeaeGenome mobilizes scientists from 14 European research institutes and 2 industrial partners from 9 countries* for a duration of 4 years (2008-2012) and a budget of 5.3 M Euros.

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Abstract reference: ITMI2009_126

High throughput SNP genotyping in wheat (Triticum spp.)

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Over the past few years, considerable progress has been made in high throughput SNP genotyping technologies largely through the investment of the human genetics community. These technologies are well-adapted to diploid species. For plant breeding purposes, it is important to see if these genotyping methods are adapted to polyploidy since most major crops are former or recent polyploids. To address this problem, we tested the capacity of the multiplex technology SNPlex™ with a set of 47 wheat SNPs to genotype DNAs of 1314 lines that were organized in four 384-well plates. These lines represented different taxa of tetra- and hexaploid *Triticum* species and their wild diploid relatives.

We observed 40 markers which gave less than 20% missing data. Different methods, based on either Sanger sequencing or the MassARRAY® genotyping technology, were then used to validate genotypes obtained by SNPlex™ for 11 markers. The concordance of genotypes obtained by SNPlex™ with the results obtained by different validation methods was 96% except for one discarded marker. Furthermore, a mapping study on six markers showed the expected genetic positions previously described.

To conclude, this study showed that high throughput genotyping technologies developed for diploid species can be used successfully in polyploids although there is a need for manual reading. For the first time in wheat species, a core of 39 SNPs is available that can serve as the basis for the development of a complete SNPlex™ set of 48 markers.

Abstract reference: ITMI2009_138

Genetic diversity of Puroindoline a, Puroindoline b, and Grain Softness Protein-1 loci in Turkish wheat cultivars

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Grain hardness plays an important role in determining both milling performance and end-use quality of wheat products. The objective of this study was to characterize allelic variations at *Puroindoline a* (*Pina*), *Puroindoline b* (*Pinb*) and Grain Softness Protein-1 (*Gsp-1*) loci in 96 wheat cultivars commonly grown in Turkey. Based on nucleotide sequence variation we found four haplotypes in *Pinb*, but only one haplotype at *Pina*. Concerning the *Gsp-1* locus, five haplotypes were detected at *Gsp-1A*, but only one haplotype at *Gsp-1B* and at *Gsp-1D* each. However, two out of five *Gsp-1A* haplotypes are new and were not published yet. These results showed that wheat cultivars commonly grown in Turkey today have a narrow genetic background for *Pina*, *Gsp-1B* and *Gsp-1D* but also harbor new alleles. This study provide useful information for improving processing quality at these loci and help to develop new strategies for wheat breeding in Turkey

Abstract reference: ITMI2009_145

Identification of differentially expressed genes in roots of wild emmer wheat genotypes contrasting in response to drought stress

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Water deficit is the major environmental factor limiting wheat productivity worldwide. The current study was aimed to identify genes expressed in roots in response to drought stress, which may be associated with drought resistance in wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* (körn.) Thell. Two wild emmer wheat genotypes contrasting in drought resistance were analysed by transcriptome analysis in response to drought stress. Analysis of variance (ANOVA) was used to test the effect of irrigation treatments (drought stress *vs*. well-watered control), genotypes (drought resistant *vs*. drought susceptible) and their interactions (G × E) on gene expression patterns. Of the 61,127 probe-sets printed on the wheat genome array (Affymetrix) 4,969 transcripts were differentially expressed between genotypes and/or treatments. Of them, 3,570 transcripts were affected by drought stress: 1,422 transcripts showed common regulation pattern in both genotypes, 3,152 transcripts were differentially expressed in the R genotype and 1,840 in the S genotype. The current study was focused on analysis of 196 differentially expressed transcripts showing three significant effects: genotype, stress and G × E interaction. Of them, 118 transcripts were differentially expressed in the R genotype whereas only 45 transcripts were differentially expressed in the S genotype. Annotation analysis of the putative gene products of the differentially expressed transcripts in the resistant genotype revealed that they are involved in multilevel regulation mechanisms, including transcription regulation (MYB, MADS-box), calcium signalling (centrin and calcium binding protein), and hormone signalling pathways (ABA, auxin, gibberellin and cytokinin). A large group of membrane structural proteins were identified as involved in compound transport (lipid, carbohydrate and peptide) and ion transport. These results suggest that some of the identified proteins are involved in adaptation to drought stress, and demonstrate the potential of wild emmer wheat gene pool for improvement of drought resistance in cultivated wheat.

Abstract reference: ITMI2009_147

Linkage map development and QTL mapping for leaf rust resistance in the model plant Brachypodium distachyon

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The model plant *Brachypodium distachyon* (L.) Beauv. has been employed to analyze the genetics of resistance to leaf rust. An F2 mapping population of 110 individuals generated between the two *B. distachyon* diploid inbred lines Bd1-1 and Bd3-1 was used to develop a molecular marker linkage map. The map was mainly AFLP-based. In addition to the initial AFLP framework, conserved markers between *B. distachyon*, barley and wheat, such as EST-derived markers, are currently being developed to be added to the map, thus providing anchor points for comparative genomics studies. To locate quantitative resistance loci on the map, the 110 F2 plants were evaluated for their reaction to the leaf rust *Puccinia brachypodii*. To improve QTL identification, F2-derived F3 families were tested for resistance to leaf rust in two independent experiments. Disease evaluations showed continuous, quantitative and transgressive segregation. Interval mapping and MQM mapping were performed on the data of the different experiments by using the software MapQTL 5.0 and then QTL positions were compared. Two major genomic regions involved in resistance to leaf rust were detected. Together they accounted for about 40-50% of the observed phenotypic variation. Our results suggest that leaf rust resistance in *B. distachyon* is a polygenic trait influenced by few major genes with large effect as observed in the *Triticeae*.

Abstract reference: ITMI2009_156

Setting up two new EMS Populations in hexaploïd wheat

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Biogemma has created a new collection of 2,000 and 5,000 plant populations from two varieties from Limagrain Verneuil Holding respectively ARCHE and APACHE. Wheat seeds have been treated with EMS at three different doses (0.6, 0.9 and 1.2%) and grown to maturity. Plants have been propagated and DNA sampled in order to set up molecular screens for candidate genes. Early steps of characterization are ongoing. We will describe the methods used to discover mutations and discussed about the rate of mutagenesis observed in these new populations. This work has been performed in the frame of "Semences de Demain" Project funded by the ministry of Industry.

Abstract reference: ITMI2009_162

Isolation and characterization of laccase gene analogues in barley (Hordeum vulgare)

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Laccases are copper-containing glycoproteins and comprise a multi-gene family in plants. Laccases belong to the group of phenoloxidases. They are very common, widely distributed oxidative enzymes. Laccases are characterized by low substrate specificity and their catalytic competence depends on the source of substrate. Laccases are able to catalyze the oxidation of various aromatic compounds with the concomitant reduction of oxygen to water. Laccases have been found in fungi, bacteria and in higher plants. Plant laccases were first proposed to play a role in lignification and in lignin degradation. Recent studies suggest that laccases perform different functions in plants and are not as genetically redundant as previously thought.

The barley laccase genes structure, DNA sequence polymorphism and gene expression have not yet been described. The aim of the project was to obtain the basic knowledge about the barley laccase-like multicopper oxidases gene family and to establish gene and allele DNA polymorphisms. Recently, new EST sequences for barley were published and eight barley UniGene clusters with partial similarity with laccase or multiccoper oxidases were used as a first approach.

Genomic sequence of the barley putative *lac* gene, covering 100% of the coding region and with high similarity to the rice and maize laccases was cloned. Genomic organisation of the putative *lac* gene with 3 introns and 4 exons and conservative domains were described. All exon/intron boundaries conformed to the GT/AG consensus. We have analysed the polymorphism of introns in a selected barley cultivars. Obtained PCR amplicons were cloned and sequenced. Data will be used for gene-specific SNP identification, for exon/intron characterisation and for laccase gene expression analyses in *Hordeum*.

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Gene-based marker development from group1 and 3 chromosomes in wheat

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Within the framework of the TriticeaeGenome project (EU-FP7, www.triticeaegenome.eu), physical maps from the wheat and barley group 1 and 3 chromosomes will be constructed and a large number of BAC end sequences will be produced. These BAC ends will represent a tremendous source of information to derive gene-based markers for the relevant chromosomes. We have developed a pipeline for automatic detection of gene-bearing BAC-ends and primer design from the wheat sequences. Amplicons are then generated on twelve wheat varieties and sequenced using the 454 technology. A first set of over 200 BAC ends has been selected from the sequences produced for the chromosome 3B physical map construction. We are also developing a pipeline for sequence assembly and HSV/SNP identification and expect to develop markers targeted for all three homoeologous copies. SNP validation will be carried out, using either the Taqman or the Illumina VeraCode technologies. Polymorphic markers will be fed back to the other TriticeaeGenome partners in order to assist the anchoring of physical contigs on the genetic map. Markers will also be used in association genetics for yield components within the project.

Abstract reference: ITMI2009_166

TILLING OF GENES RELATED TO STARCH METHABOLISM IN BARLEY

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Targeting-induced local lesions in genomes (TILLING) is a generally applicable reverse-genetics strategy providing an allelic series of induced point mutations from a population of chemically mutagenized individuals (McCallum *et al.,* 2000). At DiSTA-University of Bologna, a sodium azide-mutagenized population of barley (variety "Morex") has been developed for identifying mutations in specific genes using the TILLING procedure (Talamè *et al.,* 2008). The population, named TILLMore, has been screened for several genes based on the analysis of 8- to 12-fold DNA pools produced from M_2 or M_3 DNA samples, using LiCor and ABI-3730 sequencers. On the average 6 alleles per gene, corresponding to an extrapolated rate of one mutation every 480 kb, were identified. A TILLING service for the TILLMore resource is currently available on a cost-recovery basis and/or through collaboration (for details, see www.distagenomics.unibo.it/TILLMore/).

Starch is the major reserve of plants and the primary carbohydrate component in human and livestock diets. Mutants for biosynthetic or regulatory genes of starch metabolism often produce starch granules with abnormal morphological and molecular features that could be interesting also for technological applications.

We utilized a TILLING approach to identify mutants for genes related to starch synthesis and degradation in barley. Molecular screening for mutations has already been completed for five starch-related genes (*Limit dextrinase1*, *GBSS*I, *Bam*1, *SS*I and *SSII*) with a total number of 20 mutants identified. Almost all the mutations detected were CG-TA transitions and several (ca. 60 %) implied a change in amino acid sequence and therefore possible effects on phenotype. In four cases we identified non-sense and splice junction mutations which affect drastically the protein function. A phenotypic characterization of the mutant lines is currently in progress, results will be presented and discussed.

Session 2:
COST WG1 Divgen, Exploring and exploiting genetic diversity of the Triticeae

Abstract reference: ITMI2009_005

Genetic analysis of introgressive common wheat lines for the character awned spike

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Development of awned spike is controlled by three dominant inhibitors, *Hd* (4AS), *B1* (5AL), and *B2* (6BL). In this research, genetic control of awnedness was studied on the common wheat lines (*Triticum aestivum* L.), developed from cultivar Aurora genotype with introgressions from *Aegilops sharonensis.* The cultivar Aurora is awnless, and possesses *B1.* It could also possess a hypostatic to *B1* awn-like sprouts promoter b_n on the 6D chromosome. The spikes of *Ae. sharonensis* have awn-like sprouts. On the basis of chromosome homoeology among *Triticeae* species, the existence of awn promoter awn^P on the $6S^l$ chromosome could be presumed. Among the lines studied five were awnless, six had awn-like sprouts, and fourteen were awned. The occurrence of recessive character in the introgressive lines could be explained by *B1* gene loss after chromosome rearrangement during line development. Different phenotypes could be due to following genotypes for the 5AL and 6D($6S^l$) chromosomes: awnless lines and the cultivar Aurora have $B1B1b_nb_n$ genotype, lines with awn-like sprouts possess $B1B1\ awn^Pawn^P$ or *del del* b_nb_n one, and awned ones could be described as *del del* $B1B1\ awn^Pawn^P$. To verify this assumption, the segregation in the F_2 generation obtained from different lines crossing was studied. All F_1 hybrids obtained from awnless and awned lines crossing had awn-like sprouts. The F_2 population segregated on the awned, awnless plants, and those with awn-like sprouts according to ratios expected due to genotypes presumed. No segregation was expected in the F_2 generation from awned lines crossing; nevertheless, among 2353 F_2 plants 40 had awn-like sprouts. Taking into account the results of introgressive lines' cytological stability studying and the chromosome association patterns in meiosis M1 of pollen mother cells (PMC) in F_1 hybrids between the lines, unexpected phenotype occurrence could be explained by the meiosis abnormalities. The calculations show, that if F_1 hybrid has univalents in the meiotic M1 phase, then it produces gametes which differ in the chromosome composition. This can distort the segregation for different genes, even those ones that do not locate on the univalent chromosome, and lead to unexpected phenotypes occurrence. To conclude with, in our study the presence of hypostatic to *B1* awn-like sprouts promoter awn^P on $6S^l$ was proved and chromosome 5AL deletion in some introgressive lines was presumed.

Abstract reference: ITMI2009_006

Cytogenetical characteristic of the introgressive common wheat lines including and lacking the 4Sl chromosome

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The synthetic genome-substituted wheat line Aurosis (AABBS^{l}S^{l}) differs from genome of common wheat cultivar Aurora (AABBDD) in presence of S^{l} subgenome instead of D one. When hybrids between mentioned genotypes were obtained and self-pollinated, a number of 42-chromosome lines were developed that demonstrated some alien characters, including resistance to powdery mildew. The description of these lines concerning the number and homoeological relationship of S^{l} chromosomes that substituted ones of D genome was carried out through studying the chromosome associations in meiosis M1 of PMC in hybrids between introgressive line and cultivar Aurora, and molecular genetic marking of these lines using biochemical and microsatellite markers specific for chromosomes D and S^{l}. Among total introgressive lines studied (67) 16 possessed the gametocidal chromosome 4S^{l}, which is known for its severe gametocidal activity in the hemizygotic condition. Among 17 lines resistant to powdery mildew 11 ones included this chromosome. According to results of molecular marking, the lines studied demonstrated the presence of alien chromatin from all homeological groups, except for 2, in different combinations. Different lines had from one to three introgressions. The meiotic chromosome configurations in the hybrids between lines and between lines and cultivar Aurora were studied. On the basis of this, it could be followed out that alien genetic material in introgressive lines' genomes is presented by one (35 lines), two (16), or three substituted chromosomes (2 lines), and also one (28) or two (7 lines) translocations. The resistance of lines is related to substitution or translocation of 3S^{l} chromosome in the genome, and is controlled by a single dominant gene. According to our data, not all the lines possess the gametocidal chromosome 4S^{l}. Nevertheless, lines that do and do not posses it do not differ in such important characters as percentage of grain formation in spike, germination ability, and the number of aneuploids among progenies of introgressive lines after their self-pollination, and show the decreased values of all mentioned characters. This demonstrates that action of gametocidal chromosome in hybrid AABBDS^{l}, the progenitor of the lines developed, would influence on the progenies, even in the absence of 4S^{l} chromosome, and would complicate the transfer of the useful resistance genes (to powdery mildew in our case) on the common wheat genetic background.

Abstract reference: ITMI2009_014

Mutagenesis on diploid and hexaploid wheat

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With the development of efficient map-based cloning programs and future genome physical mapping and sequencing project, it is essential that functional tools are in place to validate gene function in wheat. This can be performed through reverse genetics where populations are established after mutagenesis and screened with candidate genes to identify knock-out mutants for each homoeologous gene copy.
To establish such reverse genetic tools for functional genomics, we irradiated seeds from the hexaploid wheat (*Triticum aestivum* L*.)* French elite cultivar Renan and produced another mutant population by TILLING on the diploid wheat *Triticum monococcum*.
These populations are currently involved in different research projects as a potential source of new alleles and for functional validation.

Abstract reference: ITMI2009_019

Molecular mapping of resistance to Fusarium head blight derived from three Triticum species

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Fusarium head blight is a serious disease in many wheat growing areas worldwide.
Apart from yield losses the contamination of the crop with mycotoxins is of major concern. Breeding for improved Fusarium resistance is plays a key role in integrated disease management. While many reports on genetic analysis of Fusarium resistance in bread wheat have been published during the past decade, only limited information is available on FHB resistance derived from wheat relatives, and from tetraploid wheats so far.
In this contribution we report about genetic analysis of FHB resistance derived from three *Triticum* sources: 1) *Triticum macha* (Georgian spelt wheat), 2) *Triticum dicoccum* (cultivated emmer) and 3) *Triticum dicoccoides* (wild emmer). Back-cross derived recombinant inbred line populations were developed from crosses of the resistance donors with well adapted cultivars. The populations were evaluated for Fusarium response in replicated experiments with artificial inoculation. The same lines were genetically analysed using SSR and AFLP markers. Map construction based in the backcross derived RIL populations was done with Carthagene and QTL mapping in Qgene. Several novel QTL were identified. In *Triticum macha* six new QTL were found on four chromosomes (2A, 2B, 5A, 5B), the largest effect QTL overlapped with the *Q-locus* (spelt type) on 5A. In *Triticum dicoccum* the largest QTL mapped to chromosome 4B (overlapping with *RhtB1*). In wild emmer (*Triticum dicoccoides*) significant QTL were detected on chromosomes 3A and 6B.

Abstract reference: ITMI2009_023

Exploring wheat genetic resources: Isolation of new resistance alleles following focused identification of germplasm strategy.

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In gene banks and seed collections, there is a large biological diversity of wheat landraces and wild relatives which remains unexplored at the molecular level. This study is a successful example of large-scale systematic allele-mining in wheat for the molecular identification of new allelic variants of the wheat powdery resistance gene *Pm3*. In wheat, race-specific resistance to powdery mildew is controlled by *Pm* genes. *Pm3,* existing in eight alleles (*Pm3a* to *Pm3g, Pm3k*), is the only wheat powdery mildew resistance gene cloned. Here, the molecular tools derived from *Pm3* haplotype studies were applied to study the genetic diversity at the *Pm3* locus. We used Focused Identification of Germplasm Strategy (FIGS) to define a subset of accessions from sites with potentially high selection pressure for powdery mildew resistance. From a virtual set of a total of 16'089 accessions, we identified 1320 accessions drawn from 323 geographical sites. These 1320 landraces were infected with a set of differential powdery mildew isolates, which allowed the selection of resistant lines. The 211 resistant landraces were analyzed at the molecular level. The candidate lines for the isolation of *Pm3* alleles, which lacked any of the known *Pm3* alleles but possess *Pm3* haplotype, were identified. The newly isolated *Pm3* alleles from these candidate lines showed sequence diversity as compared to the known *Pm3* alleles, with the differences mainly in the LRR domain. Based on transient expression assay as well as Virus Induced Gene Silencing (VIGS), we report the isolation and cloning of seven new functional *Pm3* alleles. This exploratory project demonstrates the effectiveness of the FIGS to identify a manageable and diverse set of material for screening and its potential to be applied to other important crop plants for targeting important traits or genes. The new interesting and functional alleles also enrich the genetic basis for powdery mildew resistance breeding in wheat.

Abstract reference: ITMI2009_028

Transfer of the Aegilops ventricosa gene Yr17 to wheat chromosome 2D

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According to the literature, N^v genome of *Aegilops ventricosa* is more homoeologous to D genome than to A and B genomes of wheat. Then any spontaneous transfer from N^v would be expected to occur into D genome. However the *Yr17*+*Lr37*+*Sr38* gene cluster initially carried by the translocated chromosome $6N^v$ was transferred to chromosome 2AS of the parent VPM (*Ae. ventricosa* / *T. persicum* / 3* *T. aestivum* cv. Marne) and to derived varieties as cv. Renan. We carried out an experiment to check whether the introgression systematically occurs on the A genome.

Chromosome $6N^v$ was added to the wheat complement in the rust susceptible background cv. Fidel. That addition line was crossed to monosomics 2A, 2B and 2D of cv. Courtot. Double monosomic F1 2A-$6N^v$, 2B-$6N^v$ and 2D-$6N^v$ plants were selected and backcrossed to cv. Fidel. Only one recombinant in the 2D-$6N^v$ progeny was found to carry the gene *Yr17*. That genotype with 42 chromosomes was meiotically stable. Molecular markers of the introgression in cv. Renan as well as group 2S wheat specific markers were used in order to localize the novel introgressed segment. It was found that the distal part of chromosome 2DS had been replaced by a *Yr17*-carrying *Ae. ventricosa* chromosome segment of same or similar size as that in Renan. Physical mapping is still in progress. The novel plant material offers the possibility to produce genotypes with an increased copy number of *Yr17*.

Our experiment showed that spontaneous recombination between $6N^v$ and wheat group 2 chromosomes is a rare event and that *Yr17* may be introduced either in 2A or in 2D.

Abstract reference: ITMI2009_172

WheatBiotech Project: A biotechnological network to improve competitiveness and sustainability in the Argentinean wheat chain

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Historically wheat has been one of the most important cereals in the Argentinean crops system. However, during the latest years the increase of commodities has been performed through the substitution of cereal crops (including wheat) by oleaginous crops like soybean. Such a substitution process has increased the incomes by hectares of farmers with a reduction of carbon content in soil. Extending this process in time will probably affect negatively wheat production and the sustainability of the productive environment. Under this scenario, the introduction of wheat as carbon donor in the crop rotation is critical. To get that, wheat has to be a more competitive crop in terms of yield and end-use quality. Currently, commercial wheat cultivars are developed by traditional breeding and recent advances in the fields of molecular biology, genomics, gene discovery and transformation in cereals have not been widely explored in breeding programs. All these circumstances prompted the creation of the WheatBiotech Project (WB) during 2008. WB is a network of research groups with expertise in DNA based technologies (genomics, molecular markers, transformation, VIGS), ecophysiology, end-use quality and phytopathology designed to develop and transfer technology to Argentinean seed companies, promoting a fluid communication between public and private sectors. The final goal of WB is to exploit biotechnological tools to improve the competitiveness and sustainability of the Argentinean wheat chain. The specific objectives are: (1) to characterize, evaluate and develop germplasm with increased disease resistance, yield, adaptability and end-use quality. (2) To develop molecular markers to introgress new traits into adapted germplasm by MAS. (3) To stimulate the adoption of MAS as a routine tool in breeding programs. (4) To develop protocols for transformation, VIGS and TILLING. (5) To create a web site to publicly display main activities and products. (6) To train students at different levels: MSc, PhD, short visits. (7) To promote the active interaction between different sectors of wheat chain (farmers, breeders, industry, scientists) through the organization of workshops and seminars. WB is developed by 12 partners including 7 private breeding companies from Argentina and it has been funded for 4 years starting in 2008.

Abstract reference: ITMI2009_035

Activity and polymorphism of SOD in Lithuanian barley cultivars under aluminum stress

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Aluminum (Al) toxicity can cause excessive ROS (reactive oxygen species) production in many plant species. A major safeguard mechanism against free radicals is provided by one of the antioxidative enzymes superoxide dismutase (SOD). Barley *Hordeum vulgare* L. is one the most sensitive species to Al toxicity among small-grain crops, though variation in Al resistance between cultivars does exist. SOD polymorphism and activity were determined in roots and leaves exposed for 72 h to 2 mM, and 8 mM Al^{3+} concentration at pH 4.5, 4.1, 3.9 in eight selected Lithuanian cultivars of spring barley: 'Auksiniai 3', 'Aura', 'Džiugiai', 'Ūla', 'Luokė', 'Aidas', 'Alsa', 'Auksiniai II'. According to bazal (initial) SOD activity in control plants roots the studied cultivars follows the order: cv. 'Auksiniai 3'='Ūla'>Aidas'='Alsa'>'Aura'='Luokė'>'Džiugiai'>'Auksiniai II'. The basal SOD activity in the leaves of five cultivars ('Aidas', 'Džiugiai', AII, 'Luokė' and 'Auksiniai 3') was about 2% to 11% higher than in the roots. After 72 h of Al treatment there were established changes in the activity of SOD in the barley roots and leaves. Dynamic changes of SOD activity in roots and leaves after treatment of 8 mM did not correlate with that one after treatment of 2 mM. This indicates a rather complicated response of studied cultivars to Al stress. From five to seven SOD isoforms were detected in leaves and root respectively. Al induced new isoform (SOD6) in Al stressed roots of 'Auksiniai 3', 'Auksiniai II', 'Džiugiai, 'Luokė' and 'Ūla'.
All studied barley cultivars we grouped into three groups: tolerant – 'Auksiniai II', 'Auksiniai 3', moderately tolerant – 'Džiugiai', ' Luoke ', Ūla', and sensitive – 'Aura', 'Aidas', 'Alsa'.

Abstract reference: ITMI2009_057

Association mapping of frost tolerance QTL in barley

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A better understanding of the genetics of frost tolerance could have a significant impact on world food supply, since low-temperature-related stresses limit the productivity of many plants of agronomic and horticultural value. Barley (*Hordeum vulgare*) is an excellent model system to unravel the genetic bases of frost tolerance, because of the large variation for this trait within the primary gene pool and an ever-expanding set of tools for genome analysis. Within the ERA-PG funded project ExBarDiv (Genomics-assisted analysis and exploitation of barley diversity) three different 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 within this approach, here we report the evaluation of frost tolerance in 285 barley spring cultivars. For each accession, 8 first-leaf stage plants have been cold acclimated for 4 weeks (3°C, 8 h light and 2°C, 16 h dark), then exposed to two different freezing conditions (-12°C and -10°C) for 10 h. To evaluate the effect of freezing on the functionality of the Photosystem II (PSII) reaction centers, the maximum quantum yield of the PSII photochemistry has been measured by the ratio of variable (Fv) to maximal (Fm) fluorescence in a dark-adapted state (Fv/Fm), using a Pulse Amplitude-Modulated fluorometer, after a 24 h recovery time. The same germplasm collection has been genotyped with 1536 gene-based SNPs using the Illumina™ OPA (oligo probe assay) high throughput marker technology. Molecular marker information will be used to determine the underlying population structure and perform association analyses between the phenotype and genotype data sets. Broad genomic regions containing potentially useful gene alleles for barley frost tolerance will be presented.

Abstract reference: ITMI2009_060

Characterizing registered durum wheat varieties of Turkey for some quality characteristics and pasta cooking quality related QTLs

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Bright yellow color and *al dente* cooking characteristics are two most important quality attributes in pasta products. Wheat pigments and oxidative enzymes contribute for the most part to the color of pasta, whereas wheat protein content and quality largely determine the pasta cooking quality. In this study, some quality characteristics of twenty registered durum wheat varieties grown in Turkey were investigated. In addition, they were screened for the presence or absence of pasta-quality associated gliadins and LMW glutenins through the molecular DNA markers and electrophoresis techniques. Zenit, a high-pigment Italian cultivar, and Kyle, a Canadian variety with superior pasta-cooking characteristics, were also included in the study. Several registered varieties grown in Turkey (Sarıçanak-98, Salihli, Kozmidor, Selçuklu-97 and Quashar) were comparable with Zenit in pigment and oxidative enzymes. In terms of protein quality, Gediz-75 and Kozmidor were quite similar to Kyle. In contrast, Sarıçanak, Salihli and Selçuklu-97, some of the most commonly grown cultivars in Turkey, had the g-gliadin 42 and LMW-1 type glutenins associated with weak gluten and poor pasta quality. Two of the registered varieties with high pigment content yet low protein and poor pasta quality (Sarıçanak-98 and Salihli) have been selected to improve their pasta cooking properties by transferring γ-gliadin 45 and LMW-2 glutenin encoding genes from Kyle through the marker-assisted selection technique. Results of this study will be discussed.

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Abstract reference: ITMI2009_062

Study on allele variation in loci for adaptive response and plant height and its effect on grain yield in wheat

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This study comprised the period of the modern Bulgarian wheat breeding started in the beginning of last century. The significant progress in wheat breeding practices started were assisted with the introduction of large number of imported varieties in the selection programs. Some very important productive and reproductive traits of wheat have been introduced in Bulgarian germplasm. Here we study the allele variation in: the microsatellite locus Xgwm261, linked to *Rht*8 gene and the *Ppd-D1* locus (*Ppd1*) in order to assess the temporal and geographic distribution of semi-dwarf and photoperiod response genes in Bulgarian cultivars and advanced breeding lines released in the period 1911-2007. Eight allele variants in locus Xgwm261 were identified in the analyzed germplasm collection among which only the 192bp allele is supposed to be referent diagnostic for the presence of *Rht8* gene. The *Ppd-D1a* allele was determined in almost all wheat genotypes illustrating the relationship between the photoperiod response and the adaptability to the regional environments. In this study the association between specific alleles at the *Rht-B1*, Xgwm261, *Ppd-D1, Vrn1* loci and plant height, heading time and some yield components are also statistically examined.

Abstract reference: ITMI2009_065

Establishment of a representative core set for the creation of the Spanish durum wheat core collection

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Core collections were devised as a tool for researches and plant breeders to explore and utilise the genetic diversity of germplasm collections. In Spain, four investigation groups are collaborating to create the core collection of the durum wheat collection maintained at the National Genetic Resources Centre (CRF-INIA). The objective of this work was to select 200 entries which could glean most of the genetic diversity contained in the whole collection. This subsample will be genetically characterised with molecular markers and evaluated for different traits to select about 60 accessions for the core collection,

The procedure to select the 200 entries followed these steps: i) determination of domain ii) division into genetically distinct types (taxonomic and agro-ecological regions), iii) determination of the basic unit (province) and iv) choice of the entries. Nine agro-ecological regions were established based on a cluster analysis of historical wheat yield records for the Spanish provinces. The allocation of number of accessions per group was proportional to the local varieties described in each province in the 50´s. The proportions used were logarithmic for the agro-ecological regions and taxonomic groups, and linear for provinces.

The domain was established as 579 accessions of landraces and old cultivars classified in five taxonomic groups (*T. turgidum* conv. *durum*, *T. turgidum* conv. *turgidum*, *T. turgidum* conv. *polonicum*, *T. turgidum* ssp. *dicoccum* y *T. monococcum* L).

Between four and 31 entries were allocated per agro-ecological region, and between 1 and 20 per province. Choice of entries for each province was done maximizing the diversity in agro-morphological and biochemical traits (gliadins and glutenins), and in altitude and type of soil of the collection site, if available. The einkorn accessions were selected maximizing their diversity using microsatellites.

The 200 selected entries were validated showing that the range of variation for geographic parameters (longitude, latitude and altitude) and agro-morphological characters of each agro-ecological region were included. The 200 entries are now being characterized for four gliadin loci and 39 microsatellites. The analysis of this information together with evaluation data (quality, disease resistances...) will allow choosing about 60 accessions for the core collection

Abstract reference: ITMI2009_078

Developing a multiplex set of SSR markers for the analysis of genetic resources in Brachypodium

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There is an increasing interest in *Brachypodium distachyon* as a model species for wheat, barley and temperate grasses, including the recent release of its draft genome sequence. To complement the genomic efforts, it is of great importance the collection, characterization and conservation of the genetic resources of this wild species. Although *Brachypodium* has inbreeding reproduction, a level of heterozygosity has been found in the wild individuals. Therefore, the development of inbred lines has been undertaken. We are using Simple Sequence Repeats (SSR) markers to characterize the wild genetic diversity of *Brachypodium* and to confirm the fixation of the inbreds. From previous SSR intergeneric transferability analysis across wheat-barley-*Brachypodium*, there are markers available for the species, that differ greatly in their amplification capacity across accessions and their Polymorphic Information Content (PIC). For routine germplasm characterization, the determination of a core subset with the best markers is desirable. To this aim, we have tested a set of lines representative of the genetic variability collection and determined the most suitable primer set combinations for SSR multiplexing.

Abstract reference: ITMI2009_089

Cytogenetic and molecular characterization of durum wheat chromosome transfers with 1D-associated gluten protein genes and their pyramiding

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Gluten quality of bread wheat is known to be mainly associated with high- (HMW-GS) and low- (LMW-GS) molecular weight glutenin subunits encoded by *Glu-1* (L arm of group-1 chromosomes) and *Glu-3* (S arm of group-1 chromosomes) genes, respectively, with the 1D alleles of such genes having the major impact on bread making properties.
On the other hand, durum wheat, mostly used for pasta production, is also used to prepare different types of bread worldwide. A positive effect on gluten quality was observed when durum wheat group 1 chromosomes, particularly 1A, were substituted by 1D of bread wheat. Hence, transfer to durum of 1D storage protein genes has been looked at as a means of assessing the impact of separate gene products on gluten properties, and as a possible strategy to widen the spectrum of potential end uses of durum wheat.
Transfer of chromosomal segments containing the *Glu-D1* and *Gli-D1*/*Glu-D3* loci was successfully achieved in a number of instances resorting to chromosome engineering.
Using this strategy, we isolated two 1A-1D recombinant lines, in which the *Gli-D1*/*Glu-D3* genes and the *Glu-D1d* allele (HMW-GS "5+10") were separately transferred into the 1AS and 1AL arm, respectively, of recipient durum wheat lines (named PS and PL, respectively). The recombinant nature of both transfers was initially ascertained by use of endosperm protein markers and FISH with a D-genome repeated DNA sequence as probe.
More recently, the physical structure of the recombinant chromosomes was reinvestigated by preannealing-GISH, which confirmed the terminal location and size of the 1DS segment (17% of 1AS of line PS), while showed the 1DL segment to be intercalary and subterminally located on 1AL of line PL, with a length (16% of the arm) corresponding to nearly half of the previous estimate.
Also, a detailed genetic map of both recombinant chromosome arms was developed by means of RFLP, SSR and EST markers. Some such markers were usefully employed in a further step of the work, aimed at pyramiding the 1DS and 1DL segments into the same 1A chromosome of durum wheat. Stable PS + PL double-recombinant lines have been obtained as a result of homologous recombination in the 1A portions shared by the two recombinant chromosomes present in PS x PL hybrids. Preliminary quality tests suggest that the *Glu-D3* + *Glu-D1d* combined presence could determine a slight increase of gluten quality parameters over those associated with *Glu-D1d* alone.

Abstract reference: ITMI2009_100

Ready to go into phenotyping: The wheat reference samples

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CIMMYT and its partners have committed to collect and complete the structural characterisation of diversity of the composite set in wheat. The genotypic data production of the composite set was completed in 2007 for up to 44 SSRs on a total number of 2932 entries. In order to streamline the process of evaluating genetic diversity, international reference samples of 372 spring bread wheat entries, 96 winter wheat entries, and 96 durum wheat entries were assembled based on the neutral marker information. To build the reference samples the D strategy was applied. The D-strategy has proven to maximise the representativeness of the genetic diversity in subsets of entries by including “generalists” alleles giving the advantage of minimizing population structure. The reference samples will be useful for genetic diversity studies such as SNP discovery and phenotyping agronomic traits in wheat germplasm and for analyzing the linkage disequilibrium and the association of complex traits. Single plant seed of each accession has been collected and will be deposited in the CIMMYT Wellhausen Genetic Resource Centre and are available upon request and for collaborative projects. We want to introduce the three reference samples, present their population structure and genetic diversity.

Abstract reference: ITMI2009_103

Genetic mapping of leaf rust (Puccinia hordei Otth) resistance in barley accession MBR1012 derived from Serbia and Montenegro

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To promote the effective use of barley landraces in breeding programs, they need to be fully characterised and evaluated for agronomical traits. Characterization of such genetic resources has to give special attention to disease resistance as the gene-pool of cultivated barley is largely depleted of major resistance genes for many plant pathogens, e.g. leaf rust. After detection of resistance to the available virulent isolates of pathogens, it is necessary to genetically map the corresponding genes in order to effectively incorporate them into adapted breeding lines. Here we present preliminary results on the mapping of resistance to leaf rust (*Puccinia hordei* Otth) isolates I80 and I90 detected in the landrace MBR1012 from Serbia and Montenegro. Mapping revealed the localization at two new positions on the short arm of chromosome 1H indicating two new resistance genes. Next, the relation to *Rph4*, which is also located on chromosome 1H has to be analysed by tests for allelism. Further characterization and utilisation of these genes is discussed.

Abstract reference: ITMI2009_107

Characterization of the genetic variability at MS-loci 3B chromosome in genetic pool of Ukrainian bread wheat varieties

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Characterization of genetic variation within local population and modern wheat varieties is crucial for effective conservation and exploitation of genetic resources for crop improvement programs.

The aim of this work was to characterize the level of genetic variability at MS-loci of 3B chromosome in genetic pool of Ukrainian bread wheat varieties (group A) and compare it with the level of genetic polymorphism that have been detected earlier (Chebotar et al., in press) among lines covering a large part of the existing genetic variability (group B).

Chromosome 3B carries many genes of agronomical interest such as resistance genes, pre-harvest sprouting tolerance genes, flowering time gene as well as numerous enzyme-coding genes. Earlier have been shown that the X*gwm533.2-3BS* locus closely linked to *Qfhs.ndsu-3BS* (Anderson et al., 2001), to *Yrns-B1* resistance gene (Khlestkina et al., 2002) and to *Sr2* gene (Spielmeyer et al., 2003; Ingala et al., 2008). Chebotar et al., (2005) revealed that the region of 3BS marker X*gwm533.2* was under selection pressure in breeding programs in Ukraine that lead to decreasing genetic diversity.

Using a set of 24 MS-markers located on 3B chromosome the genetic variability of 76 Ukrainian wheat varieties was analyzed. The number of alleles varied from one for locus *Xgpw7031-3B* to 17 alleles for loci *Xgpw533-3B* and *Xgwm247-3B* with an average of 6.21 alleles/locus. In total, 149 alleles were detected. Rare alleles were observed for 22 markers with an average of 2.14 alleles/locus. The PIC value for the SSRs ranged from 0 to 0.88 with mean 0.53 that is comparable to mean PIC value – 0.58 in group B.

The results confirmed the tendency towards high genetic variability at 3B chromosome among Ukrainian wheat varieties, in spite of selective pressure that have been admitted for variability of some loci.

Abstract reference: ITMI2009_110

Development of microsatellite markers in canary seed using FIASCO

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Annual canarygrass, commonly known as canary seed (*Phalaris canariensis* L.), is a self-pollinated diploid cereal (2*n* = 12) with a genome size of 3,800MB. Canary seed is presently used for bird-feed, but there is potential to develop the crop for human consumption. Canary seed starch has some unique characteristics, such as relatively high digestibility as compared to wheat, and extremely small starch granule size (2.0 µm). In the long term, yellow seeded, glabrous canary seed could eventually be used for human food, while brown seeded cultivars would be used for the traditional bird seed market. Marker-assisted selection can be used to accelerate breeding of new canary seed cultivars. Molecular markers such as, RAPD and AFLP had very limited success in differentiating canarygrass species, therefore could not be used as genetic markers for canary seed improvement. Microsatellites or simple sequence repeats (SSRs) markers generally show a high degree of polymorphism in different plant populations.

Initially, 174 barley microsatellites and 23 unigene wheat SSRs were evaluated for their transferability to canary seed. Barley and wheat SSR markers, 43 and 78%, respectively, amplified canary seed DNA, but only a few were suitable for distinguishing canary seed germplasm. FIASCO (Fast Isolation by AFLP of Sequences COntaining repeats) was used to generate microsatellite markers specific for canary seed. An enriched simple sequence repeats ($(AG)_n$, n>8) library derived from DNA isolated from a canary seed (cv CDC Togo) was produced. Analysis and DNA sequencing of library clones resulted in 700 clones from which 250 primer pairs were designed. More than 100 SSR markers amplified unique products from canary seed DNA. These SSR markers revealed the biodiversity among a panel of canary seed accessions. A mapping population is being developed for use of the SSR markers to construct a first draft genetic map in canary seed.

Abstract reference: ITMI2009_111

The impact of intra and inter specific nuclear-cytoplasmic interaction on the regulation of central metabolism in wheat.

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Breeding programs relies on the understanding of the regulation of traits. Phenotypic diversity depends on the regulation of genes at transcriptional and post transcriptional level. Thus the interaction between nucleus and cytoplasm is a key component in this multileveled regulatory network. Nonetheless, in spite of the importance of nuclear-cytoplasmic crosstalk, the mechanisms by which it affects phenotypic traits remains largely unknown. In an effort to gain further understanding on the regulation of traits in crops, we have explored the role of intra and interspecific cytoplasm regulation of central metabolism, storage reserve accumulation and gene expression in wheat. Three different alloplasmic lines were used in this work to investigate the effect of *H. chilense*, *Ae. uniaristata* and *Ae. squarrosa* cytoplasm on nuclear-cytoplasmic interaction in common wheat. Earlier works showed a clear effect of cytoplasm in these lines on agronomic traits such as anthesis timing, yield and plant height. In the present study we employed GC-MS based metabolite profiling and Affymetrix Wheat Gene-Chip® expression profiling to study the set of alloplasmic lines at the molecular level. Our results show a clear effect of cytoplasm on central metabolism of grain and leaves. We also show a differential metabolic response to low and highlight conditions as well as species associated cytoplasmic influence on metabolite content and transcript levels.

Abstract reference: ITMI2009_123

Deep phenotypic evaluation of a worldwide bread wheat core collection

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We evaluated the available genetic diversity for important agronomic traits, grain quality characteristics and rheological properties in a worldwide bread wheat core collection of 372 accessions. The traits assessed during the vegetative period were date of ear emergence, date of flowering, lodging, disease susceptibility and pre-harvest sprouting. Thousand kernel weight, test weight, grain hardness, grain protein content, pentosan viscosity and grain colour were also measured. The rheological properties of the derived white flours were estimated using mixograph and alveograph tests.

The large phenotypic variation for most of the traits studied is indicative of the wide diversity of the core collection. Several parameters (mixograph width parameters before and after peak time, alveograph dough tenacity and extensibility, near infrared measurements, like those for protein content, and absorbance measurements of palmitic acid and linoleic acid content) made it easier to discriminate between the cultivars. For example, for the alveograph tests, which are the main predictor of bread-making quality in wheat flours, the genotypes ranged from those producing very bad quality, tough, inelastic dough only suitable for animal feed, to those with excellent bread-making potential. In addition, a useful variability for important traits can be found in the landraces and old accessions illustrating the need to conserve such collections for improving grain composition. The largest ranges of variation in landraces and old cultivars rather than in more recent varieties indicates that there is sufficient variability available for those alleles which have been eliminated in breeding modern varieties.

With this phenotypic variability of wheat ''concentrated'' into a subset of manageable size, the core collection, genotyped for a large set of markers (DArTs, SSRs and SNP) could thus be considered in future prospects as a very powerful tool for association genetics studies

Abstract reference: ITMI2009_132

Linkage Disequilibrium at different scales on 3B chromosome of bread wheat

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In order to study linkage disequilibrium (LD) patterns at different scales along the 3B chromosome of bread wheat, a highly polymorphic worldwide core collection of 372 accessions was analysed using Diversity Arrays Technology (DArT), microsatellites (SSR) and SNPs markers. The analysis of population structure within the core collection sample led to distinguish five sub-populations related to the geographical origins.

At the chromosome or deletion bin scales, significant LD values were very low. Global tendency was a decrease of r^2 around 20 cM although variations were observed between sub-populations: the CIMMYT-ICARDA pool presented the longest ranged LD on the whole chromosome, spreading out to 30 cM contrary to Asian pool whose LD decreased at 20 cM. LD globally increases from old cultivars to modern varieties.

In order to study the LD at the sequence level, we used a 3 Mb contig from 3B (*rph7-fhb1* locus) which was fully sequenced and annotated. Thus, r^2 values were higher than 0.20 until 600 kb, with differences between geographical origins: 600 and 400 kb in Europe and Asia, respectively. We partially sequenced four genes of this contig (*gad1*, *hga2*, *hga1*, *hga3*) in 42 lines chosen to maximize the diversity of the core collection. The mean r^2 value of LD between *gad1* and *hga3*, separated by 725 kb, was very low with no significant value confirming the decay of LD within 600-700 kb in this region. No LD was detected between *hga2* and *hga3*, separated by only 218 kb, neither. However, despite the larger distance between *gad1* and *hga2* (about 500 kb), we observed a higher level of LD (80 LD values out of 128 >0.2). This heterogeneity may be correlated to differences of recombination rate. At the gene level, the mean r^2 values observed were higher: from 0.49 within *hga2* to 0.81 within *hga1*. Finally, we also observed LD extend variations between genes and we even observed a decay of LD within *hga2*.

To conclude, in this region and in this collection, we observed that LD dissipates over distances of 600-700 kb. This was unexpected from the evolutionary history of *Triticum aestivum* and would be favourable for association studies

Abstract reference: ITMI2009_137

Fine Mapping of the Stripe Rust Resistance Gene, YrH52, Based on Comparative Analysis with Rice, Barley and Brachypodium Genomes

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Stripe rust of wheat, caused by the fungus *Puccinia striiformis*, is one of the most destructive diseases of wheat. The wheat stripe rust resistance gene *YrH52*, derived from wild emmer wheat, *Triticum dicoccoides* accession H52, confers resistance to a broad spectrum of stripe rust races. The gene *YrH52* was previously identified and mapped on chromosome 1BS. A primary genetic map was constructed on the basis of a F_6 recombinant inbred line (RIL) mapping population, developed by crossing *T. durum* cv Langdon with *T. dicoccoides* accession H52. Using *Nor* as RFLP probe, *YrH52* gene was located on the deletion bin 1BS.sat-0.31, distal to *XNorB1* region. Markers flanking a 23.5 cM chromosome segment around *YrH52* were identified. To further delimit the location of *YrH52*, we have exploited the colinearity between rice, *Brachypodium distachyon*, barley and wheat genomes and developed new EST based cleavage amplified polymorphic sequence (CAPS) markers. Our preliminary results, based on screening of RIL mapping population indicate that our newly developed markers are located in close vicinity to the gene *YrH52*. We are currently screening a large F_2 population, with the closest markers flanking *YrH52* in order to refine the genetic map of *YrH52* and develop sub-centiMorgan map suitable for physical mapping and positional cloning of *YrH52*.

Abstract reference: ITMI2009_141

Stripe rust resistance derived from wild emmer wheat

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Wild emmer wheat, *Triticum dicoccoides*, the tetraploid ancestor of domesticated wheat, is a promising source of resistance to the stripe rust disease (e.g. *Yr15, YrH52*) caused by the fungus *Puccinia striiformis*. *Yr15* and *YrH52* are single dominant genes that confer particularly high resistance. The main objective of the current study is to clone *Yr15* using the positional cloning approach. A primary genetic map of *Yr15* was developed using a cross of a BC_3F_9 line, which contains a 1BS chromosome segment of *T. dicoccoides* carrying *Yr15*, with the recurrent parent *T. durum* cv. D447. SSR and RFLP markers were used to assign *Yr15* to 1BS deletion bin Sat0.31. ESTs assigned to 1BS Sat0.31 allowed us to establish colinearity with a 840 kb *Brachypodium distachyon* region located on Super Contig 4 and a 740 kb contig located on *Oryza sativa* chromosome 5, as well as with *Hordeum vulgare* chromosome 1HS. Further improvement of the *Yr15* map was achieved by adding retrotransposon-based (IRAP and REMAP) markers. The comparative approach enabled to narrow down the region carrying *Yr15* to 0.3 cM colinear with a ~28 kb sequence in *B. distachyon*. The sub-centiMorgan map of *Yr15* was used to identify *T. aestivum* BAC clones spanning the target region and for constructing a non-gridded BAC library from the donor line of *Yr15*. Further chromosome walking is underway to assemble a BAC contig containing *Yr15*. This study demonstrates the potential of the wild wheat gene pool for improvement of cultivated wheat.

Abstract reference: ITMI2009_148

High phytase activity : an advantage of some triticale cultivars for feeding monogastric animals

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In cereals, phosphorus is mainly under phytic acid form. However, poultry and other non-ruminants have either little or no endogenous phytase activity to hydrolyze phytate phosphorus. They have to find either free phosphorus in their diet (generally added as mineral phosphorus) or enzymes to degrade it. These enzymes can be exogenous (added to the diet) or endogenous. Cereals have themselves a more or less high level of phytase activity allowing the degradation of phytic acid. It had been shown that a high phytase activity of triticale can replace a part of the mineral phosphorus addition to the diet of poultry and enhance the phytic acid phosphorus availability for pigs. So, a high phytase activity may be useful for a better utilization of cereal phosphorus by monogastric animal and thus a better animal growth and also less phosphorous environmental pollution by excreta.

As high phytase activity of triticale could thus be an advantage for animal feeding, but also for human consumption, we studied this trait on French registered cultivars of triticale grown in trial plots in different locations during two years, in order to have a better knowledge of both the variability of the trait in triticale cultivars grown in France and the genotypic and environmental effects on this trait. Phytase activity (PA) was measured by INZO° laboratory.

These experiments showed:

- a high variability for PA (from one to three) between cultivars registered in France, (range for phytase activity was from 584 to 1810 with a mean of 1140 U/kg for 65 cultivars in 2005 and from 497 à 1642 with a mean of 944 U/kg for 67 cultivars in 2006)
- a relatively high genotypic effect but also significant year and site effects and probably some genotype x environment interactions,
- the possibility to identify low or high phytase activity cultivars.

So, this trait could be submitted to selection. If we want to know more about the genetic of this trait, crosses between high and poor phytase activity cultivars could be studied, as for example with cultivars Calao and Kortego.

Abstract reference: ITMI2009_152

New resources for wheat genetics and genomics at the John Innes Centre

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Since 2003 the UK Department for Environment, Food and Rural Affairs (Defra) has funded the UK Wheat Genetic Improvement Network (WGIN). The aim of WGIN is to develop knowledge and resources primarily to aid UK wheat breeding, but all resources are publically available to wheat researchers, Worldwide. The programme was recently renewed with a major partnership between the JIC, Rothamsted Research and the University of Nottingham, working in conjunction with a Network of UK wheat partners in Universities and breeding companies. At JIC we have, and continue to, focus on genetic and genomic resource development including the UK reference doubled haploid mapping population, Avalon x Cadenza, which now has a detailed SSR, COS and DArT based genetic map, and also has associated agronomic data from yield and other trials over several years. A fixed EMS mutant population has been developed in the elite UK spring wheat, Paragon as well as an indexed population of gamma induced Paragon deletion lines. The AE Watkins wheat landrace collection and the Gediflux Western European winter wheat collection are being characterized at the genetic level, and recently the development of libraries of Near Isogenic Lines (NILs) for key agronomic and quality traits has been initiated, as are a series of new single seed descent (SSD) populations designed for gene discovery in WGIN germplasm collections. Genomic resources consist of a set of over 1000 FAM labelled conserved orthologous set (COS) markers.

Session 3:
COST WG3 Traitgen, Deciphering agronomical traits and phenotypes in the Triticeae

Abstract reference: ITMI2009_001

Slow drought stress in relative of modern wheat

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Several studies address plant response to water scarcity by means of different genomics tool. However, knowledge is still limited due to the complexity of the stress response. We screened about 200 wild emmer wheat genotypes and then focused on twenty-six of these lines, which led to the selection of two genotypes with contrasting responses to water deficiency. Over 13,000 ESTs were sequenced from six cDNA library using leaf and root tissues of wild emmer wheat genotypes TR39477 (tolerant) and TTD-22 (sensitive) and modern wheat variety Kiziltan drought-stressed for seven days. Clustering of ESTs resulted in 2,376 unique sequences. The data obtained indicated that the genotypes shared common elements of drought stress as well as distinctly differential expression patterns that might be illustrative of their contrasting ability to tolerate water deficiencies. The ESTs generated in this study provides a new source for gene discovery in wheat.

Abstract reference: ITMI2009_007

The combination of resistance factors effective at different plant stages may explain the durability of resistance to stripe rust in the bread wheat cultivar Renan

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Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most widespread and destructive wheat diseases in areas where cool temperatures prevail. The wheat cultivar Renan, carrying the specific gene *Yr17*, has shown effective resistance for a long period of time, even though some pathotypes overcame the *Yr17* gene. The objectives of this study were to map genetic loci associated with adult-plant resistance (APR) to yellow rust in a recombinant inbred line (RIL) population derived from a cross between the resistant cultivar Renan and the rust-susceptible cultivar Récital. Field assays were performed for 4 years to score disease-progress data and identify APR QTLs. Three QTLs, *QYr.inra-2BS, QYr.inra-3BS* and *QYr.inra-6B* with resistance alleles derived from Renan were detected in 1995-1996 with the 237E141 pathotype, which is avirulent against genotypes carrying *Yr17*. These QTLs were stable and explained a major part of the phenotypic variation seen in 2005-2006, when the 237E141 V17 pathotype possessing the virulence to *Yr17* was used. Each of these QTLs contributed between approximately 4 and 15 % of the phenotypic variance and were effective at different adult plant stages. Interactions were observed between some markers of the *Yr17* gene and three Renan QTLs: *QYr.inra-2BS, QYr.inra-3BS* and *QYr.inra-6B*. Thirteen PCR-based markers of NBS-LRR resistance gene analogs (RGAs) were mapped in the RIL population Renan/Récital. Only one NBS-LRR-derived marker was linked to one Renan QTL: *QYr.inra-2BS*. Strategies for more effective control of the disease will be discussed. Resistance based on the combination of different APR types, including slow-rusting genes/QTLs and resistance factors effective at different plant stages, should provide durable resistance.

Abstract reference: ITMI2009_016

Mining for genes related to climatic stress tolerance in barley by comprehensive quantitative expression analysis

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As the first consequences of the climatic change can be observed by now, the demand for barley varieties tolerant to abiotic stress will soon increase. But as these tolerances are usually of a quantitative nature, it is essential to find the genes involved and to understand the function of these genes, before trying to breed for effective and enduring tolerance.
To this purpose three barley accessions differing in abiotic stress tolerance were set up in an assay at the exposure chambers at the Helmholtz Centre in Munich, where they were exposed to either drought stress, high UV radiation or both and one set of plants being not exposed to any stress as a control. Leave samples for RNA preparation were taken at ten different times depending on developmental stage of the plants and stress level. Four time points that cover the whole stress period were chosen for the first round of expression analysis, a 454 sequencing assay and a Agilent 44K Microarray. Based on the results of these two assays promising candidate genes will be selected and validated by real time PCR using additional time points. The results of this project are supposed to contribute to the elucidation of stress tolerance related signalling pathways as well as the identification of genes to select for in marker assisted breeding programms.

Abstract reference: ITMI2009_017

Creso x Pedroso, a new integrated DArT-SSR linkage map for dissection of agronomic traits in durum wheat

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The construction of genetic maps based on molecular markers represents the first step for the dissection of genetic basis of complex traits and for the identification of closely associated molecular markers useful to transfer the favourable alleles into elite cultivars by MAS programs.
A new genetic map of durum wheat is presented in this work, based on a segregating population derived from the cross Creso x Pedroso and consisting of 123 RILs. A total of 500 molecular markers (173 PCR-based and 327 DArT markers) were assigned to 21 linkage groups. All chromosomes were represented; for 9 chromosomes a single linkage group was identified, while the remaining five chromosomes were associated to two or three linkage groups. Globally, each chromosome was covered by a number of markers ranging from 10 to 62. The final map length was more than 1,800 cM, with chromosome 7B having the greatest coverage, with 62 markers (about 160 cM). Information on the map position of about 100 DArT markers, with unknown location, was provided, contributing towards making these markers a valuable tool for construction and comparison of genetic maps in durum wheat. The map was used to map a major gene accounting for both hypersensitivity response and partial resistance to leaf rust in Creso (*Lr14c* on chromosome 7BL); furthermore it represents a valuable tool to dissect the genetic basis of other traits of agronomic relevance as root and leaf development in early growing stages.

Abstract reference: ITMI2009_020

An ABC transporter confers durable resistance to multiple fungal pathogens in wheat

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Lr34 provides an important source of durable adult plant resistance in wheat worldwide and is expressed in maturing plants during the critical grain filling stage. The *Lr34* locus has provided durable and partial resistance to the biotrophic pathogens leaf rust, stripe rust (*Yr18*), and powdery mildew (*Pm38*) and is associated with the morphological marker leaf tip necrosis. When deployed with other adult plant resistance genes, near immunity can be achieved.

Map-based cloning and analysis of eight independent *Lr34* mutants revealed that this multi-pathogen resistance is conferred by a single gene with homology to pleiotropic drug resistance (PDR)-type ATP-binding cassette (ABC) transporters. Mutants were more susceptible to leaf rust, stripe rust, and powdery mildew, and they did not show leaf tip necrosis.

Only three sequence polymorphisms distinguished the *Lr34* allele of the resistant cultivar 'Chinese Spring' from the allele of the susceptible variety 'Renan'. The same resistance haplotype was found in the three independent breeding lineages of *Lr34*, suggesting a single evolutionary origin of the resistant allele. We have evidence that *Lr34* stimulates senescence-like processes in the flag leaf tips of resistant wheat cultivars. During senescence, nutrients such as nitrogen, phosphorus and metals are reallocated to growing seeds. Premature leaf senescence, starting from leaf tips, may therefore hamper nutrient uptake of obligate biotrophic pathogens like rusts and powdery mildew.

The observation that a multi-pathogen resistance in wheat, which comprises *Lr34*, *Yr18*, *Pm38*, as well as the phenotypic marker leaf tip necrosis, is controlled by the same gene demonstrates the existence of single genetic factors in plants which act efficiently and durably against several diseases.

Abstract reference: ITMI2009_026

Fine mapping of a durable resistance QTL against Stagonospora nodorum glume blotch in wheat

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Stagonospora nodorum is a severe necrotrophic fungal pathogen of wheat. It affects spikes and leaves of wheat causing dramatic yield losses in most cereal-growing areas of the world. Durable resistance to Stagonospora nodorum glume blotch (SNG) is quantitative, wherein several QTLs contribute to resistance. Previous studies in our research group have identified the major QTL *(Qsng.sfr-3BS)* for resistance against SNG which explained 43% of phenotypic variation and mapped on the short arm of 3B chromosome closely linked to the molecular marker SUN2-3B.
In order to isolate *Qsng.sfr-3BS* by map-based cloning, we generated a back-cross derived fine-mapping population consisting of BC_3F_2 plants. Parental lines for this population were Swiss winter wheat cultivar Arina (resistant to SNG) and Swiss winter wheat cultivar Forno (susceptible to SNG). These two varieties show strongly contrasting phenotypes in field infection tests: average artificial infection AUDPC was 2.5 for Arina and 7.3 for Forno. This clear difference in parental response to SNG would allow us to reliably score the F_2 progenies in the field. Genetically, *Qsng.sfr-3BS* has been mapped to a region of chromosome 3B, flanked by Xbarc133 and Xgwm389. Construction of the high resolution genetic map for this region is in progress with the help from the recently released 3B chromosome physical map of hexaploid wheat. According to the information from 11 physical contigs, the interval of interest is 9Mb. The newly derived ISBP, SSR markers from the physical map and SSRs we developed from respective contigs, will allow us to further increase marker density in the interest region.

Abstract reference: ITMI2009_034

Candidates of the Barley Leaf Stripe Resistance Gene Rdg2a Are Included in a Cluster of NBS-LRR Encoding Genes

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Rdg2a is a mono-mendelian barley resistance gene that confers resistance against several isolates of the seed-borne fungal pathogen *Pyrenophora graminea* (the causal agent of barley leaf stripe) and immunity against isolate *Dg2*, the most virulent isolate of a collection of monoconidial isolates.
In order to characterize the genetic basis of *Rdg2a*-mediated leaf stripe resistance, a map-based cloning approach was undertaken for this gene. For this purpose the *Rdg2a* genomic region was saturated with molecular markers developed from shot-gun sequencing of Morex BACs covering the region. Because the cv. Morex does not carry a functional allele of the resistance gene, a *5X* cosmid library of barley cv. *Thibaut* (bearing a functional allele of *Rdg2a*) was constructed. Screening of the cosmid library with markers co-segregating and tightly associated to *Rdg2a* yielded the identification of a 72Kbp cosmid contig encompassing the genomic region of the gene. Low-pass shotgun sequencing of this contig led to the identification of three sequences coding for *NBS-LRR* (Nucleotide Binding Site-Leucine Rich Repeats) proteins. Transcription analyses revealed that the three predicted genes are expressed only in the *Rdg2a*-near isogenic line (NIL) resistant genotypes but not in the corresponding susceptible NIL. The cloning of the full length cDNAs of the candidates confirmed the computational prediction for two of them, while in the third one a predicted intron is retained in the mRNA, causing a frameshift in the transcript that, most likely, lead to the production of non functional protein. Southern-blot analyses conducted on *Rdg2a*-resistant and three different susceptible genotypes as well as sequencing of the two NBS-LRR susceptible alleles highlighted genomic rearrangements in the locus suggesting that two *NBS-LRR* genes out of the three identified could represent good candidates for *Rdg2a.* Interestingly, single-cell transient assay revealed that the two predicted candidate protiens exist in the cell either inside and outside of the nuclei. The TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick end labelling) analysis of pathogen-challenged embryos of a resistant NIL revealed the absence of DNA fragmentation indicating that *Rdg2a*-mediated leaf stripe resistance may not involve programmed cell death at the host pathogen interface.

Abstract reference: ITMI2009_036

Gabi WHEAT – A whole-genome association study in hexaploid wheat (Triticum aestivum)

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Identification and mapping of quantitative trait loci (QTL) is of vital interest for marker assisted breeding in plants. One means to achieve high mapping resolution is the use of association mapping.

Association mapping offers the advantage to use collections of elite germplasm rather than requiring the creation of mapping populations, as is the case for classical QTL mapping studies.

Currently association mapping in wheat is performed using limited numbers of markers and/or traits, or focussing on the candidate gene approach only.

Here we present a project designed as a whole genome association study in hexaploid wheat, which also makes use of candidate gene information. We present the project design and first results from genotyping.

As a major result of the project we expect to find marker/trait associations. Since phenotypes are mainly evaluated by breeders, these associations will be directly relevant for field conditions. Further results of this project will be knowledge about the population structure of elite European germplasm as well as the diversity of this germplasm and the structure of linkage disequilibrium in the varieties used.

This will show if association mapping in hexaploid wheat is feasible with the microsatellite markers available today and will also yield well described material for further studies, e.g. when sufficient numbers of SNPs become available.

Abstract reference: ITMI2009_040

Towards fine mapping of the QFt.CRI-3B.1 QTL in wheat using new genic markers

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Exploiting variation for genes controlling differences in flowering time is the main way in which wheat (*T. aestivum* L.) can be adapted to different environmental conditions. Genes of small effect, commonly referred to as earliness *per se* genes, can be used for the "fine-tuning" of flowering time. A recent study (Pankova et al., 2008) identified the effect of such a QTL, *QFt.CRI-3B.1* on chromosome 3B in the Czech wheat alternative variety Ceska Presivka. This flowering time QTL was mapped on the long arm of 3B distal to marker *Xbarc164* in two populations of recombinant substitution lines (RSL) from crosses of Sandra x Sandra (CP3B) and Zlatka x Zlatka (CP3B). To narrow the QTL region for fine mapping, near isogenic lines are being produced and new Conserved Orthologous Sequence (COS) markers are being designed from ESTs located within the region. To date, forty -nine COS marker primers have been designed and screened for polymorphism using the parental varieties Zlatka, Sandra and CP. Unfortunately, none of these markers were polymorphic between these related parents. Nevertheless other mapping populations could be used to map some of these markers – doubled haploid (DH) populations for the UK wheat varieties Spark x Rialto, Charger x Badger, Avalon x Cadenza, and the ITMI RIL population, Opata x Synthetic.

To date, 3 out of 49 markers were polymorphic and mapped. Two of them (cos24 and cos30) were mapped in the Opata x Synthetic population and were distal to *Xbarc164,* and one was proximal to *Xbarc164* in the Avalon x Cadenza population. These markers will be used as anchor points for designing new COS markers using rice chromosome 1 sequence for the target parents.

Abstract reference: ITMI2009_041

Ofanto x Cappelli, an integrated DArT-SSR linkage map of durum yield for dissection of traits linked to grain yield and water deficit tolerance

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Durum wheat (*Triticum turgidum L. var. durum*) is largely grown in Mediterranean environments where drought stress affects grain yield and yield stability. Drought tolerance, high yield and yield stability are key agronomic traits characterized by a complex genetic basis, being controlled by many loci throughout the genome. This work aimed to develop a new durum wheat intervarietal genetic map based on SSR and DArT markers for the dissection of the genetic bases of important agronomic traits. 161 recombinant inbreed lines (RILs) F_8-F_9 derived from the cross between durum wheat varieties Ofanto and Cappelli were used in this study. Ofanto is a modern cultivar with yield capacity and stability; Cappelli is an old cultivar with lower yield but higher water use efficiency (WUE) with respect to Ofanto. The genetic map comprises 132 SSR, 4 TRAP and 439 DArT markers distributed within 21 linkage groups. A significant deviation from the expected mendelian ratio was registered in segregation for 13.8% of the markers. 42.9% of markers were localized on the A genome chromosomes, while 57.1% were distributed on the B genome chromosomes. The A genome accounted for a map length of 608 cM, while the B genome for 793.6 cM. The employment of the map in the dissection of physiological traits related to water stress tolerance is presented.

Abstract reference: ITMI2009_042

Genetic analysis of resistance to SBCMV in the durum wheat variety Neodur

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Soil-borne cereal mosaic virus (SBCMV), a Furovirus transmitted by *Polymyxa graminis* Led., is the causal agent of an important disease of wheat, widespread in Europe, where it causes losses in grain yield up to 70% with a great detrimental effects also on grain quality. Growing resistant cultivars represents the only effective and sustainable means of control.
Although valuable sources of resistance have been identified, little information is available on the genetic location of resistance determinants. This work aims to determine the genetic basis of resistance to SBCMV in the durum wheat cultivar Neodur by means of a bulk segregant analysis (BSA) approach. A population of 200 F_8 recombinant inbred lines (RILs) obtained from the cross between durum wheat cultivars Neodur (highly resistant) and Cirillo (highly susceptible) was evaluated for SBCMV infection severity in a field with natural inoculum sources of SBCMV, located near Bologna (Italy), during the 2007-08 season. The infection severity was evaluated by visual scoring of symptoms and DAS-ELISA assay. Ten susceptible and ten resistant lines were selected and utilised to produce susceptible and resistant DNA bulks which were analysed with more than 200 microsatellite markers polymorphic between the parents. Results of the polymorphism analysis on bulks indicate the presence of a major gene on the short arm of chromosome 2B controlling the resistant trait; detailed results and microsatellites markers suitable for transfer of the resistance will be presented.

Abstract reference: ITMI2009_043

QTL analysis of yield-related morphological traits and powdery mildew resistance in an introgressive line of bread wheat

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The introgressive line 8/1, a derivative of the susceptible spring wheat cultivar 'Tähti' and *Triticum militinae* (2n=28 A^tA^tGG), is characterized by improved resistance to *Blumeria graminis* f. sp. *tritici*. Altogether, the line carries *Triticum militinae* translocations on seven chromosomes, including translocations involved in adult plant resistance to powdery mildew on chromosomes 4A (*Xwmc*232- *Xgwm160)* and 5A *(Xgwm666-Xcfa2185)*. In the introgressive line, different agronomically undesirable effects caused by *T. militinae* translocations have been detected. QTL mapping of several morphological traits was carried out in two mapping populations developed for resistance QTL analysis: a single-seed descendant F2 mapping population from a cross of the resistant line 8/1 and cultivar Tähti (134 plants) and a doubled haploid (DH) mapping population (140 lines). Plants from both populations were evaluated for the number of ears per plant, plant height, spike length, spikelet number per 10 cm of spike, kernel weight per spike, ear-emergence time and grain free-treshing habit.

No significant effect of the 4A chromosome translocation on analysed morphological traits was detected (with the exception of slight effect on ear-emergence time) , however, the *T. militinae* allele in the Q region of chromosome 5A strongly affected several analysed traits. Recombinant DH plants carrying the resistance QTL originating from *T. militinae* and the common wheat allele in the *Q* gene region were identified.

Abstract reference: ITMI2009_047

Peroxidase gene profiling indicates a role of peroxidase genes of barley in determining level of basal resistance to rust and powdery mildew

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Class III plant peroxidases (EC 1.11.1.7; Prxs) are classical enzymes that catalyze oxidoreduction between H_2O_2 and various reductants. Generally, multiple Prxs are induced by pathogen infection, suggesting that Prxs are involved in plant defense. In plants, a high level of complexity of class III peroxidases appears to exist at the genomic level. In cultivated barley a total of 105 unigenes have been identified. We applied Motif-directed Profiling in two barley mapping populations (L94 x Vada and Vada x SusPtrit) to estimate the number of prx gene clusters in the barley genome and establish their possible association with QTLs for basal resistance to *Puccinia* rust fungi and to powdery mildew (*Blumeria graminis* f.sp. *tritici*).

Six degenerate primers were developed on the basis of two conserved prx motifs (FHDCV or VSCADI). With twelve primer-enzyme combinations, a total of 168 polymorphic bands could be mapped to an integrated barley linkage map. The distribution of these prx-based markers was irregular, but similar in the two mapping populations. The highest number of markers occurred on Chrom 1H and 7H and fewest on Chrom 4H. A re-sampling procedure of the data suggested that the barley genome contains about 40 prx clusters.

QTLs for resistance to cereal rusts and powdery mildew fungi were considered to co-locate with prx-based markers if their peak marker mapped in the same BIN as at least one prx-marker. For all three resistances considered, viz. basal resistance to *P. hordei*, basal resistance to *B. graminis* f.sp. *hordei* and non-host resistance to *Puccinia* rusts of several other grass and cereal species, we found a statistically significant association between the QTLs and the prx-markers. Such associations were not significant between prx-markers and other agronomic traits, nor between resistance QTLs and other expressed sequence-derived markers. This indicates that the association was not due to the occurrence of gene-rich areas on the barley genome.

We conclude that prx profiling is effective in finding the genetic location of prx genes, and that QTLs for basal resistance to rusts and mildew fungi tend to locate with prx clusters, making prx-related genes prime candidates to explain natural differences in levels of basal resistance.

Abstract reference: ITMI2009_048

Effects of alleles of dwarfing genes on the morphometric parameters of the kernels of bread wheat

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The reduction in plant height from the standard to semidwarf levels is known to be associated with significant increasing in kernel number, harvest index and eventually with higher grain yield for bread wheat. The pyramiding of the dwarfing genes (*Rht8c* + *Rht-D1b*, *Rht8c* + *Rht-B1e*, *Rht8c* + *Rht-B1d*, *Rht8c + Rht-D1b+ Rht-B1d*) in the genotypes of modern Ukrainian bread wheat varieties have been detected (Chebotar et al., 2007).

In order to investigate possible effects of alleles of dwarfing genes on morphometric parameters of the kernels the nine bread wheat analogue lines were tested. On the genetic background of historically most popular local varieties series of the analogue lines genetically different in plant height were created by V.V. Khangildin in Plant Breeding and Genetic Institute (Odessa). Their allelic composition related to principal dwarfing genes *(Rht8, Rht-B1, Rht-D1*) have been estimated by using PCR-analysis and test for sensitivity to gibberellic acid: Kooperatorka (*Rht-8a, Rht-B1a, Rht-D1a*) - Kooperatorka K-90 (***Rht-8c**, Rht-B1a, Rht-D1a*) and Kooperatorka K-70 (***Rht-8c**, **Rht-B1e**, Rht-D1a*); Odesskaya 3 (*Rht-8a, Rht-B1a, Rht-D1a*) - Odesskaya 3 K-75 (***Rht-8c, Rht-B1b**, Rht-D1a*); Odesskaya 51 (***Rht-8c**, Rht-B1a, Rht-D1a*) - Odesskaya 51 K-73 (***Rht-8c**, Rht-B1a, **Rht-D1b***); Stepnyak (*Rht-8₂, Rht-B1a, Rht-D1a*) - Stepnyak 2K (***Rht-8c**, Rht-B1a, **Rht-D1b***). As a control were used lines of Bezostaya 1, Karlik 1, Odesskaya semidwarf. The one hundred kernels for each line were scanned and the morphometric parameters of the kernels: *l* - major and *d* - minor axes length, *l*/*d* - compactness, *P* - perimeter and *S* - square of projection of kernels to a surface area of scanner where calculated using computer program ImageJ 1.41 (NIH, USA). Significance of differences was tested by Student's criterion at P=0,05 and P=0,01.

There have been detected increasing of kernels compactness from 4,7% to 21,6% for analogue lines comparatively to level of recurrent parental lines (P=0,01), the only exception was analogue line Odesskaya 3 K-75. We consider these differences as side effects of single alleles of dwarfing genes and their combination. The influence of alleles of dwarfing genes on the other morphometric parameters of the kernels is discussed.

Abstract reference: ITMI2009_051

Modification of the cell wall pectin to improve wheat defence response to fungal pathogens

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In several plant-pathogen interactions, the plant cell wall represents the main barrier to penetration and/or colonization of the host tissue. Because of this, its reinforcement should increase plant resistance. One way to reach this goal and test this hypothesis is the modification of the pectin component of the cell wall. Pectin is secreted in a highly methylesterified form and is demethylesterified *in muro* by pectin methylesterase (PME). The activity of PME is regulated by specific protein inhibitors (PMEIs). Since highly methylesterified pectin can be less susceptible to hydrolysis by enzymes such as fungal endopolygalacturonases (endo-PGs), the inhibition of endogenous PME by PMEI might increase pectin resistance to degradation by fungal PGs. In order to verify this possibility in wheat, a number of wheat lines expressing the pectin methylesterase inhibitors AcPMEI (from *Actinidia chinensis*) has been produced. This inhibitor is active on endogenous PME and transgenic lines showed a reduced PME activity. No obvious phenotypic differences between the transgenic lines and the wild type plants have been observed, however, the degree of methylation is higher in the transgenic compared to the wild type plants. Moreover, transgenic tissue is more resistant to digestion by fungal PGs and transgenic plants showed a significant reduction of symptoms following the infection with the fungal pathogen *Bipolaris sorokiniana*. Infection experiments with the floral pathogen *Fusarium graminearum* are also in progress.

Abstract reference: ITMI2009_052

The polygalacturonase-inhibiting protein 2 (PvPGIP2) limits disease symptoms caused by fungal pathogens in transgenic wheat plants

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The plant cell wall represents the main barrier to the colonization of host plant tissue. To overcome this obstacle, most fungal pathogens produce a variety of enzymes that degrade the wall polysaccharides. Endo-polygalacturonase (PG) is secreted during the infection process and the relevance of its activity has been demonstrated in several plant-pathogen interactions, including the cereal pathogens *Claviceps purpurea* and *Fusarium graminearum.*

PG activity is controlled by the protein inhibitor PGIP (PG-inhibiting protein). The effectiveness of PGIP in limiting cereal tissue colonization by fungal pathogens has been recently shown in transgenic wheat plants expressing the bean PvPGIP2. These plants showed a significant reduction of symptoms following the infection of *Bipolaris sorokiniana* or *F. graminearum* suggesting that pectin hydrolysis is an important step for fungal penetration of cereal species in spite of a low pectin content in their cell wall.

In order to verify better the contribution of PGIP in wheat defence, we have analyzed in more detail the response of wheat transgenic lines expressing PvPGIP2 against *F. graminearum* and we have extended the analysis to different pathogens, including *Claviceps purpurea* and *Puccinia recondita*.

Abstract reference: ITMI2009_055

A roadmap for zinc trafficking in the developing barley grain based on laser capture microdissection and gene expression profiling

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Nutrients destined for the developing cereal grain encounter several restricting barriers on their path towards their final storage sites in the grain. In order to identify transporters and chelating agents that may be involved in transport and deposition of zinc in the barley grain, expression profiles have been generated of four different tissue types: the transfer cells, the aleurone layer, the endosperm, and the embryo. Cells from these tissues were isolated with the 'laser capture microdissection' technology and the extracted RNA was subjected to three rounds of T7-based amplification. The amplified RNA was subsequently hybridized to Affymetrix 22K Barley GeneChips. Due to the short average length of the amplified transcripts and the positioning of numerous probe sets at locations more than 400 base pairs (bp) from the poly(A)-tail, a normalization approach was used where the probe positions were taken into account. On the basis of the expression levels of a number of metal homeostasis genes, a working model is proposed for the translocation of zinc from the phloem to the storage sites in the developing grain.

Abstract reference: ITMI2009_056

The NAC transcription factors of barley and their role in leaf senescence

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Transcription factors of the NAC family are found exclusively in plants, where they are apparently implicated in the regulation of many diverse functions including senescence, biotic and abiotic stress tolerance as well as developmental processes. In the genomes of *Arabidopsis* and rice more than a hundred members of the NAC superfamily have so far been found. However, as of now there are only a very few for which the exact regulatory function is known. Each member of the NAC superfamily is characterised by having a highly conserved N-terminal NAC domain, which includes a DNA binding motif, and a very variable C-terminal transcriptional activation domain.
In this work, we focus on the NAC transcription factors in *Hordeum vulgare* (barley), a commercially important cereal and a well-explored model for the small-grained cereals. Although the barley genome is not yet sequenced, extensive EST sequences are available, which have been exploited in this project to establish a provisional family of barley NAC transcription factors. So far this family comprises 39 putative NAC genes in *H. vulgare*, all based on their homology to known NAC genes from other organisms available in public databases. Phylogenetic analysis of this family demonstrated that the 39 members represent all of the major groups of the NAC superfamily. There are, however, some smaller groups of *Arabidopsis* or rice NAC genes, for which there are no available *H. vulgare* homologues.
The leaf senescence process of crop plants is important in relation to efficient remobilisation of nutrients, in particular nitrogen, to the grain during maturation of the crop. A number of NAC genes have been associated with senescence in different plant species, and our aim for the barley NAC transcription factors is to investigate their putative role in the regulatory, interrelated molecular networks underlying leaf senescence in barley. So far, real-time PCR gene expression studies have shown that several of the barley NAC genes are up-regulated during senescence. Furthermore, by comparing the expression levels of each gene over a number of different tissues, a few of these genes stand out as being very likely involved specifically in senescence regulation. We are currently generating transgeneic barley lines, in which two selected NAC genes are either over-expressed or knocked down, in order to facilitate further investigations of their regulatory targets.

Abstract reference: ITMI2009_075

Analysis of transgenic wheat with improved powdery mildew resistance

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In wheat, race-specific resistance to the fungal pathogen powdery mildew (*Blumeria graminis* f. sp. *tritici)* is controlled by *Pm* genes. We are studying one of these genes, *Pm3*, which has eight functional alleles (*Pm3a-g* and *Pm3k*) and is a member of the large class of CC-NB-ARC-LRR disease resistance genes. The *Pm3* alleles were previously cloned and functionally confirmed by single cell transient transformation assays. For the *Pm3a-g* alleles, we have now developed transgenic lines expressing a single, epitope-tagged PM3 derivative driven by the constitutive, strong ubiquitin promoter. These transgenic lines will help to answer fundamental and applied research questions about the use of a major resistance (*R*) gene as transgene: In field trials, we are testing for transgene resistance function under field conditions, and we will determine the fitness costs of a single *Pm3* gene when overexpressed. Since single major *R* genes are rapidly overcome in lines with widespread cultivation, we are testing if mixtures of transgenic lines carrying each a different *Pm3* allele represent a long term agronomical strategy to increase *R* gene durability. Results from the field trial in 2008 and preliminary data from the field season 2009 will be presented.

Abstract reference: ITMI2009_081

Functional analysis of the wheat ortholog of OsGW2, an E3 ligase potentially involved in grain development

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Crop grain yield is an important agronomically complex trait determined by various components as grain weight and number of grain per spikelet, and controlled by quantitative trait loci (QTLs). To date, several wheat grain yield QTLs were identified as loci *QYld.crc-2B* (McCartney et al., 2005) and *QTgh.ipk-7D* (Röder et al., 2008), but none was functionally characterized. Recently a new QTL, GW2, controlling rice grain width and weight has been cloned and characterized in *Oryza sativa* (Song et al., 2007). *OsGW2* encodes a new RING-type E3 ubiquitin ligase that is involved in the Ubiquitin Proteasome 26S (UPS) pathway. This proteolytic pathway is found in a wide range of life processes, including embyogenesis, hormone signalling, and senescence. A loss of function of OsGW2 increases cell number in rice grain spikelet hull and so seems to be a novel negative regulator of cell division. In this study, we identified the OsGW2 ortholog in wheat, *Triticum aestivum* cv Récital, which shares 87% amino acid sequence identity. Moreover, the previously unknown RING-type domain is conserved. A TaGW2 amino acid sequence comparison in *Triticum aestivum* and wheat diploids genotypes indicated a perfect RING domain conservation but variations in the target protein recognition site in the C-Terminal part. Expression pattern analysis of *TaGW2* mRNA showed that it was expressed constitutively in vegetative tissues and seeds. However, it appeared varying over grain development. Wheat GW2 *in vitro* autoubiquitination and yeast two-hybrid assays showed that it has an E2-dependent E3 ubiquitin ligase activity as OsGW2.

RNAi and overexpression experiments have been undertaken to identify the GW2 function in wheat grain development. A TaGW2 natural variants research in a range of wheat varieties (INRA Clermont-Ferrand, wheat core collection) is also in process in order to link TaGW2 function to natural grain size.

McCartney *et al*. 2005 Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452 x AC Domain. Genome 48:870-883

Röder *et al*. 2008 Fine mapping of the region on wheat chromosome 7D controlling grain weight. Funct Integr Genomics 8:79-86

Song *et al.* 2007 A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nature genetics 39:623-630

Abstract reference: ITMI2009_090

Intragenic allele pyramiding combines different functional specificities of wheat Pm3 resistance alleles

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Plant resistance (*R*) genes induce effective defence responses against pathogens containing matching avirulence (*Avr*) genes. Alleles of the CC-NB-ARC-LRR encoding *R* gene locus *Pm3* of wheat (*Triticum aestivum*) confer resistance to a differential subset of races of the fungal pathogen wheat powdery mildew (*Blumeria graminis* f.sp. *tritici*). It was investigated whether race-specificity determinants of different *Pm3* alleles can be combined to create resistance genes with broader resistance spectra. Therefore, we first characterised the specificity of *Pm3* alleles on the molecular level. Avirulence analyses of powdery mildew populations identified two natural examples of a broad- and its corresponding narrow-spectrum *Pm3* allele: *Pm3a* has a resistance spectrum which covers the one of *Pm3f*, but extends towards additional races. The same is true for the pair *Pm3b* and *Pm3c*. The molecular analysis of these allelic pairs revealed a novel role of the ARC protein domain as enhancer of *Avr*-dependent resistance reactions. Sequence analysis of the *Pm3a* to *Pm3g* alleles and a series of recombinant *Pm3* genes identified single, specific, solvent-exposed residues in the predicted concave part of the C-terminal LRR motifs as the main determinant of allele specificity. Variable residues of the N-terminal LRR units are necessary, but not sufficient, to confer resistance specificity. Based on these data, a chimeric gene was constructed which combines all polymorphic sites of *Pm3d* and *Pm3e*. This construct showed the combined resistance specificity of the parental alleles, confirming that it is possible to create genes with broader functional specificity by intragenic allele pyramiding.

Abstract reference: ITMI2009_091

Physiology, molecular biology and disease resistance of barley necrotic mutants nec1 and nec3

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Plant necrotic (lesion mimic) mutants show constitutive hypersensitive response, necrotic lesions on various plant parts and elevated expression of pathogenesis related (*PR*) genes and reactive oxygen species even in absence of disease. Necrotic mutants also often exhibit altered resistance to different pathogens. Thus, there is a link between plant hypersensitive response and disease resistance, which makes necrotic mutants a useful tool for understanding mechanisms of hypersensitive response and potentially for improving crop plant disease resistance. Barley *nec1* and *nec3* mutants belong to an initiation class of necrotic mutants. *Nec1* gene has been cloned and its structure has been characterized, while cloning of *Nec3* gene is still in progress. Despite the advances in molecular characterization of these mutants, the exact causes of necrotic phenotype are not clear and the phenotype itself is only broadly defined. We previously showed that *nec1* mutant was characterized by a reduced stomatal aperture, however, the guard cells were still regulated by abscisic acid. Here, we show that unlike the *nec1*, in the *nec3* mutant stomatal opening is not affected. Both mutants displayed an increase in expression of the *PR1* gene compared to the parental varieties, however, only the *nec1* mutant showed increased expression of the antiapoptotic gene *BI-1*. In addition, *nec1* plants showed 3-fold increase in H_2O_2 concentration in seedling leaves. The observed substantial physiological and gene expression differences between *nec1* and *nec3* mutants suggest that different steps of the hypersensitive response pathway are affected. Parkland, parental variety of the *nec1* mutant, carries no known powdery mildew (*Blumeria graminis* f.sp. *hordei*) resistance genes. Real - time PCR data suggested that expression of the *Nec1* gene in Parkland was not changed during infection with a mixed population of powdery mildew 24 h, 48 h and 72 h after inoculation. Data on mildew resistance of the *nec1* mutant will be reported.

Abstract reference: ITMI2009_092

Genetic and deletion mapping of phytoene synthase Psy2 gene on group 5 chromosomes of durum wheat

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Yellow pigment content is one of the criterion in the assessment of semolina quality of durum wheat and is of particular importance in determining the commercial and nutritional quality of end-products such as pasta. A yellow to amber colour is generally preferred by consumers rather than a brown or cream one. Semolina colour is the result of carotenoid pigment content of the grain and of their residual content after the storage of the grain or semolina, of the carotenoid oxidative degradation by lipoxigenase during processing, and of processing conditions. The biosynthetic pathway of carotenoids involves more than ten enzymatic steps, among which the step catalyzed by phytoene synthase (Psy), dimerizing two geranylgeranyl pyrophosphate molecules at the beginning of the pathway, is assumed to be rate-limiting in the carotenoid biosynthesis. Duplicated *Psy* genes (*Psy1* and *Psy2*) in 12 species (including common wheat) from eight subfamilies of the grass family were identified by Gallagher et al (2004), and *Psy1*, but not *Psy2*, exhibited a strong association with yellow pigment content of endosperm. A recently identified *Psy3* gene in maize was found to be highly expressed in embryo and roots particularly in response to abiotic stress. Characterization of *Psy* genes and the development of functional markers for them are of importance for marker-assisted selection in crop breeding. In wheat the genes *Psy1* were mapped on the group 7 chromosomes, and found co-segregating with QTLs for phenotypic variation for endosperm colour. In this study, the *Psy2* gene was mapped on group 5 chromosomes using a segregant population of 121 progenies derived by crossing the durum wheat cultivars Latino and Primadur characterised by low and high values of yellow pigment content, respectively. Nulli-tetrasomic, ditelosomic and deletion lines of hexaploid wheat cultivar Chinese Spring were used to assign PCR fragment to chromosome bins. The *Psy-B2* locus co-segregated with a 5B QTL, demonstrating an association of this gene with phenotypic variation for endosperm colour.

Abstract reference: ITMI2009_094

Quantitative trait loci (QTL) associated with adaptation to Mediterranean dryland conditions in the barley cross Arta x Keel

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A novel barley recombinant inbred population derived from the Syrian landrace Arta and the Australian cultivar Keel was tested for agronomic performance under drought. The population was phenotyped at two different locations in Syria over three consecutive years with a winter and spring planting in two years. The derived population showed extensive transgression for yield in the driest environments. A QTL mapping using 100 SSR markers revealed QTLs for yield and yield component traits, where both parental lines improved traits at different loci. Morphological and developmental differences were at the basis of marker by environment interaction effects for yield. Identification of QTL affecting field performance of barley under drought stress is a first step towards the understanding of the genetics behind drought tolerance in the field.

Abstract reference: ITMI2009_097

Gene expression analysis of related wheat lines with contrasting levels of head blight resistance after Fusarium graminearum inoculation

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Fusarium head blight (FHB) is one of the most destructive diseases of wheat worldwide. In this research work we aim to identify expressed genes involved in FHB resistance of wheat, in particular to identify genes/alleles associated with the two major resistance QTL Fhb1 and Qfhs.ifa-5A. The tools used for identification of target genes are cDNA-AFLPs and Affymetrix microarrays.

Eight spring wheat genotypes with contrasting phenotypes for FHB resistance were used in this study: the highly resistant line CM82036, the highly susceptible cultivar Remus, four BC5F2 near isogenic lines (NILs) for Fhb1 and Qfhs.ifa-5A and two doubled haploid (DH) lines from a CM82036/Remus mapping population differing in Fhb1 and Qfhs.ifa-5A. At anthesis the lines were challenged by F. graminearum or water. The inoculated spikelets were harvested at several time points after inoculation and dissected for RNA preparation.

For the cDNA-AFLPs 256 AFLP primer combinations were applied, resulting in a total of about 13,000 gene tags evaluated. We identified 430 (3.3%) pathogen-responsive transcript derived fragments (TDFs), most of these TDFs were induced after Fusarium inoculation in the two parental lines and the two DH lines. 202 of these pathogen-responsive TDFs were also detected analyzing the transcript profiles of the NILs. Among the 13,000 TDFs investigated, only 47 (0.36%) were differentially expressed after Fusarium inoculation and CM82036- or Remus-specific. Ten (0.08%) TDFs were differentially expressed between the DH lines and were associated with Fhb1 and Qfhs.ifa-5A. Surprisingly no pathogen-responsive and NIL-specific transcripts were identified. Only 12 (0.09%) constitutively expressed TDFs were polymorphic between the NILs.

111 TDFs were excised, cloned and sequenced. Sequence analysis of the gene tags revealed homologies to wheat genes involved in e.g. defence response, metabolic pathways (phenylpropanoid pathway), in regulatory functions, transport function. For 26 gene tags cDNA-AFLP expression patterns were confirmed by quantitative real-time PCR analysis.

As a complementary approach to the cDNA-AFLPs we used microarrays. The Affymetrix wheat GeneChip experiments were carried out in cooperation with the University of Minnesota. The bioinformatics analysis of the Wheat GeneChip experiments using R BioConductor software is in progress. The comparative analysis of the data on candidate genes gained by the two approaches will be presented.

Abstract reference: ITMI2009_104

Towards Fine Mapping of Cdu1, a Major Gene Regulating Cadmium Accumulation in Durum Wheat Grain

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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 (*Triticum turgidum* L. var *durum*) cultivars have a genetic propensity to accumulate Cd in grain to levels exceeding proposed international limits of 100 ppb. Genetic differences in Cd uptake are most closely associated with differences in Cd root-to-shoot translocation. A single QTL for Cd uptake has been reported on 5B, but the genetic factor(s) conferring the low Cd phenotype are currently not known. Genetic mapping of grain Cd concentration at two environments coupled with chromosome localization in aneuploid stocks indicated that the gene(s) associated with variation in Cd concentration, designated *Cdu1,* resides in wheat bin 5BL9 0.76-0.79. Using composite interval mapping, we have resolved the QTL to a 2.5 cM interval flanked by two expressed sequence tags (ESTs). The closest EST mapped near (0-0.3 cM) the center of the QTL and explained 80% of the phenotypic variation in grain Cd concentration. This EST has similarity to an ABC-like transporter and is a potential candidate for *Cdu1* as ABC transporters have been associated with intracellular Cd sequestration in *Arabidopsis*. We have used these ESTs to anchor the durum genetic map to the rice and *Brachypodium* genomes, which will allow us to identify additional molecular markers for further saturation and fine mapping of the region. Our ultimate goal is to achieve sufficient saturation to allow positional cloning of *Cdu1* in durum wheat.

Abstract reference: ITMI2009_115

GABI RYE-FROST: Exploiting allelic and phenotypic diversity for frost tolerance in winter rye

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Rye (*Secale cereale* L.) has been shown to be the most frost tolerant small grain cereal species and thus could serve as a model crop for frost tolerance in cereals. The project aims at the exploration of allelic and phenotypic diversity for frost tolerance, a major prerequisite for genetic improvement of this trait. Focussing on the target trait objectives of the project are i) understanding the genetic basis of frost-tolerance in rye, ii) high-resolution association mapping of candidate genes contributing to frost tolerance, iii) analysing the LD within and between selected candidate genes and iv) identifying alleles of superior interest for marker-assisted breeding.

The plant material is derived from five Eastern European winter rye populations as well as one Middle European population pre-tested for frost tolerance. Since the rye populations are self-incompatible, up to 68 plants of each population were crossed to the same self-fertile inbred line and a total of 204 S_0 plants were obtained. Each S_0 plant represents one gamete of the source population. S_0 plants were cloned and selfed seed was harvested to obtain sufficient seed of each S_1 line for phenotypic and genotypic analyses. To evaluate frost tolerance, 140 S_1 lines and a set of 16 inbred lines were phenotyped in 2007/2008 in controlled and semi-controlled environments as well as in field trials. In 2008/2009 204 S_1 lines and 16 inbred lines were phenotyped.

On the molecular level, population structure was determined using 37 SSR markers distributed across the rye genome. PCR assays were established for candidate genes which are known to be involved in the reaction of the plant to frost stress. Candidate gene sequences are analysed in the S_0 plants and inbred lines for five members of the *CBF* transcription factor family, as well as for *VRN1* (AP1 domain), *COR14b*, and *ICE2*. Identification of SNP and/or InDel polymorphisms between S_0 lines is in progress. An association genetics approach will be used to identify functional polymorphisms associated with frost tolerance.

Abstract reference: ITMI2009_116

A new tool to facilitate a positional cloning approach using barley morphological mutants (NILs)

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Barley combines the characteristics of a model organism for genetic studies and genome analysis in cereal crop species with the fact of representing itself an important crop species for the worldwide agriculture. The seven chromosomes of barley represent the basic genome of Triticeae species. With about 5.3 Gbp of haploid genome size it contains the smallest genome among the crop species such as wheat and rye. Since many traits are conserved among the highly collinear genomes of the Triticeae, the smaller and diploid barley genome serves as a model for Triticeae crop genome analysis.

Our goal within the BARCODE project, is to isolate at least one gene responsible for a morphological phenotype using both a traditional forward genetics and a bioinformatic approach. A unique series of available Bowman NILs mutants (Franckowiak J., 1996) has been genetically mapped using an high throughput gene-based SNP genotyping platform in order to define the boundaries of the introgressed segments based on their unique SNP haplotypes. Three mutants were selected from this NILs collection for further analysis. To progressively narrow the genetic interval to a small genetic/physical distance that will achieve the required genetic resolution for positional cloning we developed new molecular markers and screened large F2 cross populations segregating for the prioritized phenotype. In addition, a detailed comparative gene content map of the lesion-containing introgressed segments was compiled by comparative genome sequence information (synteny to rice) for each isoline locus, and the gene expression profiles were mined in different Triticeae public access databases. This combined analysis provided, for each mutant phenotype, a list of candidate genes. Region containing candidate genes will be sequenced both in the mutants and in the wild type, and comparatively examined in order to identify potential polymorphisms that cause the deviation of the phenotypes. We also plan to analyze expression profiles of candidate genes in mutant and wild type, and if necessary initiate their functional characterization also by use of specific available tools (RNAi or VIGS).

Abstract reference: ITMI2009_122

Construction of subtractive cDNA library and identification of wheat (Triticum aestivum L.) transcripts induced by brown rust (Puccinia triticina)

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The goal of the project is to identify wheat transcripts involved in signaling and resistance against brown rust. We focused on *Lr*9 gene because it provides effective and durable resistance against this pathogen.

To isolate the transcripts that are up regulated upon pathogen infection we used two isogenic lines: susceptible cv. Thatcher and resistant Tc*Lr9* line. RNA extracted from plants 12, 20, 26, 32 and 44 hours after inoculation with *P. triticina* urediniospores was used for cDNA synthesis and construction of suppression subtractive hybridization SSH cDNA library with BD PCR-Select cDNA Subtraction Kit (Clontech). The whole procedure was performed in forward and reverse orientation in order to identify wheat genes induced and repressed by the pathogen in the resistant line. The final products of two subtractive hybridizations and nested PCR were cloned in pGEM-T vector, transformed to *E.coli* JM109 and selected as the SSH forward (357 clones) and SSH reverse (436 clones) library.

The macroarrays (the membranes loaded with cDNA-inserts) were differentially hybridized with DIG-labeled forward and reverse cDNA. Hybridizations of the forward library revealed that 111 clones were pathogen-induced. Up to now, 64 clones were sequenced and subjected to preliminary bioinformatic analysis. Significant part of the clones (nucleotide as well as amino acid sequences) revealed high homology to transcripts / proteins of known or putative involvement in the response and/or resistance against the pathogen.

Currently we analyze the expression pattern of the selected clones and set up the experimental bases for VIGS-based functional analysis. The selection of the clones and the design of the VIGS experiments are partly based on our earlier results on wheat-rust interaction concerning the pattern of H_2O_2 accumulation - oxidative burst, micronecrosis and the inhibition of the pathogen growth.

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Abstract reference: ITMI2009_133

Why is wheat yield not increasing any longer in France and Europe

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After a dramatic increase from 1940 to 1990, wheat yield seems to have reached a plateau. By using linear regression of national or regional yields over sliding 15-years windows from 1952 to 2007 we found the slope of the regression to be 0 since 1992.
One explanation which is often put forward is that the genetic variability for yield traits has been exhausted. To test this hypothesis, we have estimated to main genetic effect in three independent series of multi-years trials. Using a mixed model approach, we were able to estimate the theoretical yield that the best 20 cultivars in each series would have had in an "optimal" reference year. We showed that the corrected average yield fits quite perfectly a single regression with a slope ranging for 0.09 to 0.1 $t^{-1}year^{-1}$ according to the series.
In 1992, the European Union changed its system of public support to agriculture, moving from a deficiency payment per t to a standard subsidy per hectare. This new system was supposed to less encourage over-production. However, all economic studies showed no clear trend of input reduction and even less a rupture in 1992. For example, statistics show that the total amount of N application continued to increase until 2000-2001, while yield stagnation had already started. Then it decreased by about 20 Kg $NO3.ha^{-1}$. But meanwhile, a better fractionation was increasingly used, with a reduction of early application and thus an increase of the overall Nitrogen fertilizer efficiency. Similar results were found for other inputs, particularly for fungicides.A detailed study was then carried out on climatic factors that are supposed to limit yield. Eco-physiologic models were used to simulated yield (of a constant genotype) using the registered climatic conditions in several regions. Most simulations showed yield reductions since about 1990, due to higher occurrences of climatic limiting factors. It should be noticed that wheat experiences more frequent occurrence of limiting factors, despite a global trend to earlier development stages (harvest time is about 2 weeks early than in the past). It is generally admitted than climate will continued to warm up, thus the frequency of limiting factors is expected to be still increasing. Finally, the synthesis of our different studies led us to estimate that about 70% of the yield stagnation should be explained by climate change, the other 30% having a multi-factorial origin.

Abstract reference: ITMI2009_136

Proteomics analysis and chromosomal assignment of wheat endosperm albumins and globulins using the deletions lines of cv Chinese Spring.

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The albumins and globulins of wheat endosperm each account for approximately 10% of total flour protein. They are soluble proteins, mainly enzymes and proteins involved in cell functions. Characteristics of the kernel storage components, flour composition and dough properties are resulting of the endosperm enzymes. Studies have been carried out to identify major enzymes present in the endosperm both using classical biochemical and molecular tools. Beside the storage proteins which are well known, some enzymes were identified and mapped using proteomic approach with the ITMI segregating progeny (Merlino M. *et al*, 2009). But many albumins and globulins which are not segregating remain unknown and unassigned. In using the deletions lines of the cultivar Chinese Spring, we present here results of chromosomal assignment of the genes encoding these albumins and globulins, occurring in the mature endosperm. The 70 deletions lines and 1 ditelosomic line of cv Chinese Spring were analysed by 2DE (IPG strip pH 3-11, 24 cm), with four replicates. Approximately 700 spots were detected on Coomassie stained gels. Image analysis of the 285 2DE gels was performed in using the Progenesis Samespots software. In the frame of the Healthgrain European program, proteomic map of Chinese spring was achieved in collaboration with DTU laboratory in Denmark. In using Mass Spectrometry and data mining, about 400 proteins were identified. Image analysis allowed us to locate genes encoding many proteins on chromosomal segments. In addition to the presence/absence characteristics, quantitative variations of spots volume were detected and statistical analysis was performed. This led us to hypothesis some possible cis and/or trans regulations of genes controlling the protein quantity of several spots.

Reference:
Merlino M, Leroy P, Chambon C and Branlard G (2009) Mapping and proteomic analysis of albumin and globulin proteins in hexaploid wheat kernels (*Triticum aestivum* L.). Theor Appl Genet, DOI 10.1007/s00122-009-0983-8.

Abstract reference: ITMI2009_139

Genetic analysis of the kinetics of monocarpic leaf senescence in winter wheat (Triticum aestivum L.)

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Senescence is a highly regulated process in plants. In monocarpic plants such as wheat, it allows remobilization of nutrients (nitrogen in particular) from senescing leaves to other organs, especially the developing grain. This process is under genetic control and may also be highly influenced by environmental conditions such as water and nitrogen availability. The dynamics of senescence influence both nitrogen and carbon assimilation through its rate and duration. It might have contrasting effects on the allocation of these two elements to the grain due to the negative relationship between grain yield and grain protein concentration. On one hand, a slower and/or longer senescence ("stay-green" phenotypes) is associated with a longer photosynthetic assimilation and a higher grain yield while on the other hand, a faster and/or shorter senescence ("early senescent" phenotypes) is associated with a reduced yield but a higher grain protein concentration. Moreover, some particular environmental conditions might require adapted ideotypes and therefore particular attention should be paid on this process in plant breeding. It is therefore necessary to unravel the genetic basis of monocarpic senescence.

The objective of this work is to identify genetic markers linked to QTL controlling senescence kinetics parameters through a multi-environment QTL analysis carried out on one mapping population. This population was grown for two years in France and in the UK. In 2009, two nitrogen levels were applied to assess the effect of nitrogen fertilization on senescence kinetics. Fungicide, insecticide, and herbicide treatments were applied on both N levels to achieve an optimised management of the crop. Senescence scoring was made visually three times a week on the penultimate leaf in Clermont-Ferrand (France) and at the canopy level in the other locations. These data were then fitted with an equation describing senescence as a two phase process. A genetic map was established using mainly SSR and DArT® markers.

The results highlight the effect of nitrogen fertilization on senescence kinetics and the main QTL identified for senescence parameters showing potentially interesting chromosomal regions to unravel the genetic basis of this process.

Abstract reference: ITMI2009_142

Phenotyping individual progenies of barley lines for yield and homeostasis at the whole plant level

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Accurate and precision phenotyping is a prerequisite for dissecting the molecular nature of polygenic traits and increasing the efficiency of breeding programs. The effectiveness of phenotyping methods, particularly for traits like yield and stability of performance, is reflected in their ability to reliably differentiate among very similar genotypes under real field conditions.
The documented negative correlation between yielding and competitive ability *(Euphytica 50:57-62, 1990),* if not properly considered, represents a major obstacle towards the goal of accurate field phenotyping. Another important decision concerns the use of appropriate selection designs. The honeycomb selection designs *(PBR 13:87-139, 1995; FCR 75:191-209, 2002)* are designs specifically conceived for evaluating single plants in the absence of the masking effects of intra-plant competition, providing for reliable estimation of agronomical parameters through multiple replicates (>> 30) and unique layout properties that effectively eliminate the negative effects of soil heterogeneity.
Data will be presented of two years of field phenotyping individual progenies within local barley lines for yield and homeostasis at the whole plant level, which led to the isolation and classification of genotypes that differ with respect to quantitative traits, representing a series of valuable genetic material for dissecting agronomical traits at the molecular level.

Abstract reference: ITMI2009_154

Genetic and molecular characterisation of the Rht8 locus in bread wheat

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The use of dwarfing genes to reduce plant height, improve lodging resistance and increase grain yield has been one of the major strategies in developing modern bread wheat cultivars. Dwarfing and semi-dwarfing genes are common in wheat varieties, but many of them have a negative impact on yield. *Rht8* is one of the few, together with the Green Revolution genes, to reduce height without penalising yield. Besides being associated with a 10% height reduction, it is also favoured in dry environments such as the Mediterranean countries as it has no effect on coleoptile length or seedling vigour. With the recent climate changes, the use of *Rht8* could become increasingly important even in more Northern latitudes. Therefore, the characterisation of *Rht8* will provide invaluable tools to further the understanding of height regulation and offer new potential benefits to breeding programmes of bread wheat.

In the present study, 89 single-chromosome recombinant lines between chromosomes 2D of *Mara* (*Rht8*) and *Cappelle-Desprez* (wt) in a homozygous *Cappelle-Desprez* background, are used to test new gene-based markers around the *Rht8* locus and to perform comparative genomics studies of the region in the model species *B. distachyon* and *O. sativa*. This approach led to the identification of 11 new markers flanking the *Rht8* locus, spanning 2.8Mb in *B. distachyon* and 3.6Mb on rice Chr4. To narrow the genetic interval further, selected single-chromosome recombinant lines carrying *Rht8* were crossed with *Cappelle-Desprez* to result in an F_2 population of more than 10,000 individuals. Recombinant F_2 plants were identified through high-throughput fluorescent genotyping and the resulting F_3 families will be scored for height to implement the fine mapping of *Rht8*.

Rht8 is classified as a gibberellin (GA)-sensitive dwarfing gene because mutant plants respond to the exogenous application of GAs, but its role, if any, in GA metabolism or signalling remains unknown. It has been reported that *Rht8* has no effect on leaf elongation rate or leaf responsiveness to the application of GAs. Surprisingly, the response has never been studied before in elongating wheat culms, the organs that determine plant height. Interestingly, the endogenous GA content is not significantly different between *Rht8* mutant and wt plants. This novel physiological evidence will be discussed and linked to the fine mapping data to gain a deeper understanding of the *Rht8* locus.

Abstract reference: ITMI2009_157

Association Mapping of Fusarium Head Blight in a French winter wheat population

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Fusarium head blight (FHB) is a destructive disease of wheat in Europe. However, with few exceptions, the genetic bases of the resistance are still obscure. The objective of this study is to better characterize the FHB resistance of the French wheat germplasm through association mapping. Linkage disequilibrium can be used for identifying associations between traits of interest and genetic markers.

A panel of around 200 elite lines was evaluated for FHB resistance during two seasons. Spray and single-spikelet inoculations were applied. The severity, incidence and spread of the disease and morphological characters putatively involved in such mechanisms of resistance were assessed by visual scoring.

This study used mapped diversity array technology (DArT) markers and SSR markers to find associations with traits linked to Fusarium Head Blight resistance. Integrated maps and a high density genetic map containing around 600 DArT markers and 200 other markers are currently under development. Several linear mixed models will be used to assess marker-trait associations incorporating information on population structure and covariance between relatives.

The first results show that a part of the associated markers were found in genomic regions where previous reports had found genes or quantitative trait loci (QTL) influencing the same traits, providing an independent validation of this approach. In addition, many new chromosome regions for disease resistance were identified in the wheat genome.

Abstract reference: ITMI2009_165

Mapping Eyespot Resistance Genes in Wheat

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Eyespot is an economically significant fungal disease that infects cereal crops, causing lodging, premature ripening of grain and reduced crop yield. In the current study we have developed a series of PCR-based markers to facilitate selection of both the major eyespot resistance genes, *Pch1* and *Pch2,* in wheat breeding programmes. The potent resistance gene *Pch1*, transferred from *Aegilops ventricosa*, is located on the distal end of the long arm of chromosome 7D. The RFLP marker *Xpsr121* and the endopeptidase isozyme allele *Ep-D1b* have previously been shown to be closely linked to *Pch1.* This effect is probably due to reduced recombination in the region of the introgressed *A. ventricosa* segment. Simple sequence repeat (SSR) markers were integrated into the genetic map of a single chromosome 7D recombinant population segregating for *Pch1*. Sequence-tagged-site (STS)-based assays were developed from the RFLP marker *psp121* and a 7DL wheat EST that contains a SSR. SSR markers WMC14 and BARC97 and the *Xpsr121*-derived marker co-segregated with *Pch1* in the recombinant population.

The gene *Pch2* derives from the variety Cappelle Desprez and has been located to the distal end of chromosome 7AL. A single chromosome (7A) recombinant population segregating for *Pch2* was screened for eyespot resistance and mapped using SSRs. QTL interval mapping closely associated *Pch2* with the SSR marker WMC525. To further investigate *Pch2* resistance, cDNA-AFLP was used to identify genes differentially expressed between the eyespot susceptible variety Chinese Spring (CS) and the Chinese Spring chromosome substitution line Cappelle Desprez 7A (CS/CD7A) containing *Pch*2. Induced and constitutive gene expression was examined to compare differences in gene expression between non-infected plants and plants infected with *Oculimacula acuformis*. Of approximately 4700 fragments generated, only 34 were differentially expressed between CS and CS/CD7A. Clones were obtained from 29 fragments and sequences from these were characterised according to their homology with known nucleotide and protein sequences. Interestingly, four had homology to proteins involved with plant defence responses. PCR primers were designed for each fragment and, using SSCP analysis, three of these mapped in the region of *Pch2* making them candidates for involvement in eyespot resistance. Taken together with the results from previous reports, the results from the present study strongly suggest that *Pch1* and *Pch2* are homeoloci.

Abstract reference: ITMI2009_167

Network studies of gene expression responses to water stress in durum wheat

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Molecular genetic, genomic, bioinformatic, statistical and physiological tools are employed to understand and identify allelic variation, candidate genes and loci defining responses and yield adaptation to drought in durum wheat. A mapping population from two breeding lines, Lahn (high yield potential) x Cham1 (drought adaptation) was studied in 27 field trials in Syria, Tunisia, Morocco and Italy. Statistical analysis has identified RILs showing stable yield under drought for further genomic studies using the wheat Affymetrix expression arrays. ANOVA and C-means soft clustering analysis of time-series gene expression data has revealed a high level of structure in leaf transcript responses to water stress. Mapping to orthologous proteins and subsequently to metabolic pathways has identified genes and transcription factors involved in early and late responses to water stress. We have correlated genes and transcription factors and established possible regulatory networks.

Session 4:
Genome Structure and Evolution

Abstract reference: ITMI2009_008

Paleogenomics in cereals for trait improvement

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In an attempt to unravel the structure and evolution of the cereal ancestor genome we have re-assed the synteny and duplications of the wheat, barley, rice, maize and sorghum genomes to to identify and characterize shared duplications. We combined the data on the intra-genomic duplications with those on the colinear blocks and found duplicated segments that have been conserved at orthologous positions since the divergence of cereals. By conducting detailed analysis of the length, composition, and divergence time of the conserved duplications we identified common and lineage-specific patterns of conservation between the different genomes that allowed us to propose a model in which the grass genomes have evolved from a common ancestor with a basic number of five chromosomes (90 MYA) and then twelve chromosomes (60 MYA) through whole genome duplications (tetraploidization) and translocations followed by lineage specific segmental duplications, chromosome fusions and translocations (Salse *et al.* 2008).
Based on these data an 'inner circle' comprising 5 ancestral chromosomes was defined providing a new reference for the grass chromosomes and new insights into their ancestral relationships compared to early marker-based macrocolinearity studies between the grass genomes that have led to arrange their chromosomes into concentric 'crop circles' of synteny blocks (Bolot *et al.* 2009). The established cereal ancestor genome structure in term of chromosome structure and gene content offered the opportunity to study the impact of evolutionary shuffling events such as polyploidizations on (i) genome structure (witch mechanism drives the diploidisation process); (ii) gene expression (role of epigenetics on neo/sub functionalisation); (iii) agronomical trait genesis (role of whole or segmental genome duplications on QTL epistatic interactions), that will be discussed in details (Throude *et al.* 2009).

Salse J, *et al.* (2008) Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. Plant Cell. 20(1):11-24.
Bolot S, *et al.* (2009) The 'inner circle' of the cereal genomes. Current Opinion in Plant Biology**.** 12(2):1-7.
Throude M *et al.* (2009) Structure and Expression Analysis of Rice Paleo-Duplications. Nucleic Acids Res. 37(4):1248-59.

Abstract reference: ITMI2009_012

CACTA DNA-transposon Caspar evolution across wheat species through sequence analysis and comparative in situ hybridization

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DNA transposons (Class II transposable elements) move in genome using cut-and-paste mechanism via DNA-intermediate. The main contribution into bulk of DNA-transposons makes CACTA subclass accounting for about 10% of large wheat genomic sequences characterized to date. Among CACTA the Caspar family is the one well-represented and frequently found in wheat BAC-clones originated from different chromosome regions. However, for several Caspar probes there was shown FISH hybridization to distal chromosome regions. Nowadays the available information about wheat large genomic sequences, including our data of BAC_2050O8 sequencing, enables us to particularly investigate the evolution of Caspar family across different Triticeae species and its role in speciation of subtelomeric regions.

In the present study we combined two approaches: (1) phylogenetic analysis of different Triticeae Caspar-like elements taken from BAC_2050O8 and from TREP and NCBI databases; (2) comparative *in situ* hybridization of specific probes, derived from 4BL subtelomeric Caspar_2050O8 element, on chromosomes of polyploid wheats and their diploid progenitors.

The comparative FISH analysis showed that Caspar_2050O8 localized mainly on the subtelomeric chromosomal regions, with some inter- and intra-specific differences in *Aegilops* and *Triticum* species. We observed the strongest hybridization of *Caspar*_2050O8 in the translocation region of *T. durum* and *T. aestivum* involving chromosome arms 4AL, 5AL, and 7BS.

The phylogenetic analysis of amino acid and nucleotide sequences coding for transposase, and dot-plot pattern of sub-terminal sequences showed the clear clustering of Caspar-like elements into three major groups: the elements originated from *Hordeum vulgare* formed distinct branch on the tree, and the elements belonging to *Triticum* and *Aegilops* species fell into two groups, corresponded to highly relative Caspar and Clifford families. Some of these Caspar-like elements originated from non-subtelomeric regions, so the specific FISH pattern is associated with increased elements concentration in these subtelomeric loci rather than sequence divergence between elements.

Therefore, Caspar-like families of transposable elements are predominant in subtelomeric chromosome regions of polyploid wheats and their diploid progenitors; the increase of copy number toward the telomeres may be related to the high recombination rate in the distal regions.

Abstract reference: ITMI2009_022

Survey of Sucrose-Phosphate Synthase Gene in Bread Wheat to Study Sequence Polymorphism and Genetic Diversity

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In plants, sucrose plays important metabolic roles like transporting sugar, as storage reserve, as solute and as a signal compound. Sucrose phosphate synthase (SPS) is a main regulatory enzyme involved in sucrose biosynthesis pathway in many crop plants, including wheat. Present study was designed to study the structure of SPS genes in wheat. However, complex genome and presence of the genes in duplicate/triplicate sets, located in the same chromosomal region of homoeologus chromosomes made the study very difficult and highly challenging. Genome specific primers were designed for SPS genes using exon anchored primers that could amplify one or more introns, which are supposed to be more polymorphic than the exons. Each of the individual SPS loci of the three wheat A, B and D genomes was studied. Approximately, 4kb of each genome could be compared and analysed. Detailed investigation of introns and exons was conducted. Sequences alignment was conducted to search for the SNPs present in each of the three genomes. So developed SPS genome specific primers are being used for the PCR amplification in multiple accessions of *T. aestivum*, *T. turgidum* subsp*. durum*, *T. urartu, Ae. speltoides* and *Ae. Searsi.* Sequence polymorphism, separately for each wheat species, will be grouped into distinct haplotypes. Genetic mapping and genetic diversity studies in common wheat, for SNP sites will also be conducted using either pyrosequencing or direct sequencing. Linkage disequilibrium analysis will also be performed.

Abstract reference: ITMI2009_024

Cytogenetic and molecular genetic analysis of the Aegilops variabilis Sv chromosomes carrying resistance to nematodes in wheat

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The allotetraploid species *Aegilops variabilis* Eig (2n=28, UUS^vS^v) belongs to the tribe Triticeae and is closely related to wheat. One accession, *Ae. variabilis* n°1, was found to be resistant to the cereal cyst nematode (CCN) and the root knot nematode (RKN). As the genetic variability for resistance to those two pests is limited within wheat, this accession was crossed to bread wheat. Previous work enabled the development of two addition lines and two translocation lines carrying resistance. Here, we demonstrate using genomic *in situ* hybridization (GISH), that there is no U-S^v interchange in the parental accession of *Ae. variabilis*. However there are multiple rearrangements in the S^v chromosomes. The *Ae. variabilis* chromosome carrying the gene *CreX* for resistance to CCN combined segments with homoeology to wheat groups 1, 2, 4 and 6. The *CreX* gene belongs to the group 1 part and it was likely to have been introduced into chromosome 1B at a similar location as the previously found QTL *QCre.srd-1B* for CCN resistance. The second *Ae. variabilis* chromosome carrying *CreY* and *Rkn2* combined segments with homoeology to wheat groups 2, 4 and 7 on its short arm and 3 on its long arm. It was designated as $3S^v$. The two genes for resistance are carried by its long arm and have been transferred to wheat chromosome 3B through homoeologous and genetically balanced recombination. Different SSR markers present in the introgressed segments could be used in marker-assisted selection.

Abstract reference: ITMI2009_061

Structure of the Triticum genome: sequence clustering by codon usage

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The variation in comparative frequency of codons in different coding sequences is often used as a factor when the evolution speed and conservativity of the sequence is evaluated.
Quantification of codon usage biases is associated with various biological factors such as gene expression level, gene translation initiation signal, gene length, protein and amino acid composition, protein structure, tRNA abundance, mutation frequency and patterns, and GC content.
It has been shown that codon usage can be used as a factor for the classification of coding sequences. Here we structure the genome of *Triticum aestivum* L. using the codon usage of the known sequences. The results from the pairwise codon usage comparison are used to build a hierarchical structure of the genome. The clustering of the results is used to search for groups with distinct functional or structural properties.

Abstract reference: ITMI2009_082

Identification of a novel non-autonomous DNA transposon associated with the dehydration-responsive TdDRF1 gene in durum wheat and other triticeae species.

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The analysis of several sequences of the *Triticum durum* Dehydration Responsive Factor 1 (*TdDRF1*) gene, consisting of four exons and three introns, from various wheat species, put in evidence the presence of a DNA transposon element. Initially, we have found this non-autonomous transposon element in durum wheat, inserted between intron 1 and intron 3 of the gene. Subsequently, it has been identified in *Aegilops speltoides*, *Triticum urartu* and other triticeae species, due to the nature of synteny in these species.

This non-autonomous DNA transposon shows many standard transposon signals, such as a terminal inverted repeat (TIR-18bp), target site duplications (TSD-2bp-TC), many internal inverted repeats and a variable number of tandem repeats.

This non-autonomous transposon does not code for a transposase enzyme and its peculiarity is that it is located inside the *TdDRF1* gene. Inside the transposon there are two small transcribed regions (the exon 2 and the exon 3 of the gene, respectively) and they can be subjected to alternative splicing. In theory, if the transposon moves, these transcripts will be carried together into the target destination genomic region.

Our efforts are focused on understanding the possible role of this non-autonomous DNA transposon in the alternative splicing regulation through a deep genomics approach. Some interesting hypotheses should be validated: for example, this transposon could be suppressed by DNA methylation or by RNA interference. In particular, the hypothesis on RNAi is very attractive because *TdDRF1.2* transcript has a premature termination codon (PTC), which could trigger the nonsense mediated decay (NMD) regulation pathway.

Abstract reference: ITMI2009_085

Evolution of the Yr15 region in the Poideae

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The *Yr15* locus resistance to stripe rust, a disease caused by caused by the fungus *Puccinia striiformis*, is being approached by a positional cloning strategy. The source of the allele is wild emmer wheat, *Triticum dicoccoides*, which is the tetraploid ancestor of domesticated wheat. The recently determined seqeunce of the wild grass *Brachypodium distachyon* proved a great aid in finding markers sufficiently close to *Yr15* to permit selection of BAC clones covering the locus. This grass, due to its small genome size and phylogenetic position basal to the key agricultural Poideae, is proving a useful tool for the Triticeae. We were able to narrow down the region carrying *Yr15* in *Triticum* to a syntenic ~28 kb sequence in *B. distachyon*. Chromosome walking within wheat enabled identification of BAC clones corresponding to the region of *Yr15*. The repetitive fraction of the genome, in particular the transposable elements, is generally the fastest evolving. In view of the genome size difference between *Triticum* and *Brachypodium* we expected large changes to have occurred in this locus. Here, we examine the transposable element complement of the *Yr15* locus and discuss evolution of the locus since the divergence of the last common ancestor of *Triticum* and *Brachypodium* within the Poideae. We make reference as well to othologous regions from the rice and other genomes.

Abstract reference: ITMI2009_095

New satellite DNA sequences from Leymus

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The genus *Leymus* (lymegrass) comprises about thirty polyploid perennial grass species in the tribe Triticeae (family Poaceae). The main distribution of *Leymus* is in the temperate regions of Eurasia and North America. Its natural habitats range from coastal to inland areas, including diverse soil types and climatic conditions. The plant's tolerance to environmental stresses such as salinity and drought has made lymegrass attractive for wheat breeding. Desirable agronomic quality has been transferred from lymegrass to wheat crops via hybrids and triteymus amphiploids. In order to make full use of lymegrass genetic resources, i.e. in the targeted transfer of agronomic traits to wheat crops and to obtain advanced amphiploids for marginal and sustainable agriculture, the *Leymus* species and their genomes must be characterized. Previous studies indicated a large diversity in the *Leymus* genome and therefore the aim of this study was to isolate new repetitive DNA sequences that can be used for differentiating *Leymus* species and elucidating their genomic relationships. A C_0t-1 DNA plasmid library was generated from genomic DNA of American tetraploid species *L. triticoides*. A family of highly repetitive satellite DNA sequences, designated Lt1, was obtained from this library. The Lt1 family consisted of 380-bp *Sac*I repeating units arranging in tandem arrays. A 120-bp *Msp*I subfamily was discovered within this family, indicating that cytosine methylation may have played an important role in the evolution of satellite sequences. The Lt1 satellite was localized in the subtelomeric heterochromatic blocks of *L. triticoides* chromosomes, which are present on all chromosomes and often on both arms. The Lt1 sequences are abundant in *L. triticoides*, but absent in its closely related species *L. racemosus*. Significant homology was found between the Lt1 family and numerous repetitive sequences from Poaceae species, indicating that the Lt1 is an ancient family of tandemly repeated sequences in grasses.

Anamthawat-Jónsson K, Wenke T, Thórsson ÆTh, Sveinsson S, Zakrzewski F, Schmidt T (2009) Evolutionary diversification of satellite DNA sequences from *Leymus* (Poaceae: Triticeae). *Genome* 54(4): 381-390.

Abstract reference: ITMI2009_119

Detailed analysis of four genes from the Rad51 gene family in bread wheat

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Recombination plays a major role in determining the structure and evolution of eukaryotes. A better understanding of its underlying mechanisms can help improving the genetic basis of crop species and controlling more efficiently the introgression of favourable alleles in breeding programs. In wheat (*Triticum aestivum*), it was shown that recombination increases gradually with the distance from the centromere. However, we have no idea of the genetic variability of genes involved in this complex process. In this study we have cloned and analysed the sequences of four genes belonging to the *Rad51* family involved in DNA repair and recombination namely *Rad51*, *Rad51B*, *Rad51C* and *Xrcc3*. Existing sequences were identified in the wheat EST databases using those from the model species (Arabidopsis, rice). Primers were designed from these sequences for amplification of the coding region of the four genes. The three homoeologous copies from the A, B and D genomes were isolated using the Chinese Spring BAC library and the sequences were compared. Specific primers were designed for each copy when possible and used for chromosomal assignment using wheat aneuploid stocks. These primers will be used for expression analysis of the genes as well as for genetic variability screening in a wheat core collection. These latter results will be discussed.

Abstract reference: ITMI2009_128

Comparative study of group 7 chromosomes in wheat and barley

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Our project is working towards development of a physical map and genome sequence for chromosome arms 3S and group 7 chromosomes in collaboration with the European Seventh Framework Programme. We are evaluating the physical distance between markers along these chromosomes, assessing the gene content and investigating recombinational behaviour in wheat and barley.

Physical mapping generates crucial data for map-based cloning of particular genes and is a preliminary step to the sequencing of large and repetitive genomes such as wheat and barley. Chromosome 7H of barley carries important loci controlling traits such as malting quality, disease resistance, salt tolerance and drought tolerance. We have identified QTLs related to yield under drought stress and salt tolerance on chromosome 7A of wheat. Our goal is to assemble a physical map across 7H and compare it to genetic maps of the orthologous wheat chromosomes.

A physical map of chromosome 7H is being constructed using whole genome libraries in collaboration with the IPK (Germany) and by locating expressed genes to 7H using large insert clones. The wheat ESTs previously mapped on deletion bins of group 7 chromosomes are anchored onto BAC clones of Morex and assembled into contigs. As the physical map is assembled across 7H and the gene containing regions are identified, the full gene content of the clones will be determined through sequencing. Shotgun sequencing of isolated 7D chromosome arms will also be undertaken to identify the gene content of these arms and the data used to support the anchoring of the genetic and physical maps. The second objective is to identify the orthologous regions on all group 7 chromosomes by building a comparative map between the barley physical map and the wheat genetic map. We are focussing on a high recombination region located on short arm of chromosome 7A. The barley physical contigs will be tied onto the wheat genetic maps of chromosome 7A by developing SNP markers from EST sequences and by using large recombinant inbred lines populations available at the ACPFG.

Abstract reference: ITMI2009_155

Breakpoint localization of a reciprocal translocation present in Albacete

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'Albacete' is a Spanish six-row barley variety with a relatively long cycle and an alternative growth habit. It is drought tolerant showing relatively stable grain yield production. For these properties, it has been grown for decades, particularly in drought-prone areas. 'Albacete' is the only extensively grown barley variety that it is known to have a reciprocal chromosomal translocation. Linkage relationships in a translocation heterozygote are altered as recombination between loci usually is significantly reduced, particularly within the interstitial segment and between loci close to the translocation breakpoints (TBs). Our recent results using molecular data suggest that chromosomes 1H and 3H are involved in this translocation. The markers found to be closely linked to the TB are HvM20 and EBmac501 for chromosome 1H and a small group of genomic SSR markers for 3H, which were placed around the centromere.
In order to find the precise physical location of the TBs in Albacete, we followed two different approaches: (i) 1H and 3H wheat-barley telosome addition lines to determine the physical positions of the above genetic markers by PCR, and (ii) Fluorescence *in situ* hybridization with 18S-5.8S-26S rDNA probe and microsatellite probes ($(ACT)_5$, $(CAG)_5$) to visualize the chromosome translocation.

Session 6:
Molecular breeding

Abstract reference: ITMI2009_002

Detection of SSR Markers Linked with Gene(s) controlling Components of Yield Traits in Durum Wheat under Drought Stress and Non-stress Conditions

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Grain yield and associated agronomic traits are important factors in durum wheat (*Triticum turgidum* L.) improvement. In this study, microsatellite (SSR) markers related to components of yield such as 1000 grain weight (TGW), grain weight per spike (GWS), number of grains per spike (GNS), spike number per m^2 (SN), spike weight (SW), spike harvest index (SHI) and harvest index (HI) were assessed. F_3 and F_4 lines derived from 151 F_2 individuals developed from a cross between a drought tolerant (Oste-Gata) and a drought susceptible durum wheat genotypes (Massara-1) were used. Data of investigated traits were collected from four environmental conditions including two irrigation regimes of drought stress at terminal growth stages and normal field conditions in two growing seasons. Two hundred microsatellite markers reported for A and B genome of bread wheat were used and 30 of them were polymorphic. QTL analysis and marker identification were conducted following genome-wide single marker regression analysis (SMA) and composite interval mapping (CIM). The results of SMA revealed that about 20% of phenotypic variation of harvest index and 1000 grain weight could be explained by *Xcfd22*-7B and *Xcfa2114*-6A markers in different environmental conditions. Similarly, markers *Xgwm181*-3B, *Xwmc405*-7B and *Xgwm148*-3B and marker *Xwmc166*-7B were found to be associated with spike harvest index and grain weight per spike, respectively. A total of 20 minor and major QTL were detected, 5 for 1000 grain weight, 2 for average grain weight, 2 for grain number per spike, 3 for spike number per m^2, 5 for harvest index, 2 for spike harvest index and 1 for spike weight. All of these QTL were near 10 different markers. Also, some of these QTL were prominent and stable under drought stress and non drought stress environments and explained up to 49.5% of the phenotypic variation.

Abstract reference: ITMI2009_004

Mapping of quantitative trait loci for resistance to spot blotch caused by Bipolaris sorokiniana and the stay green trait in wheat (T. aestivum L.) lines 'Ning 8201' and 'Chirya 3'

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Spot blotch caused by *Bipolaris sorokiniana* is a destructive disease of wheat in warm and humid wheat growing regions of the world. To identify quantitative trait loci (QTLs) for spot blotch resistance, two mapping populations were developed by making the crosses between common susceptible cultivar 'Sonalika' with the resistant breeding lines 'Ning 8201' and 'Chirya 3'. Single seed descent (SSD) derived F_6, F_7, F_8 lines of the first cross 'Ning 8201' × 'Sonalika' were evaluated for resistance to spot blotch in 3 blocks in each of the 3 years. After screening of 388 pairs of simple sequence repeat (SSR) primers between the two parents, 119 polymorphic markers were used to genotype the mapping population. Four QTLs were identified on the chromosomes 2AS, 2BS, 5BL and 7DS and explained 61.9% of phenotypic variation in a simultaneous fit. The QTL on chromosome 2A was detected only in one year and explained 22.7% of phenotypic variation. In the second cross ('Chirya 3' × 'Sonalika'), F_7 and F_8 population were evaluated in 3 blocks in each of the two years. In this population also, four QTLs were identified on chromosomes 2AS, 2BS, 2DS and 7DS. The QTLs identified in the 'Chirya 3' × 'Sonalika' population explained 34.4% of phenotypic variation in a simultaneous fit. The alleles for reduced disease severity in both the populations were derived from the respective resistant parent. The QTLs *QSb.bhu-2B* and *QSb.bhu-7D* from both populations were placed in the same deletion bins, 2BS1-0.53-0.75 and 7DS5-0.36-0.61, respectively.

In addition to spot blotch, the parents, 'Chirya 3' and 'Sonalika' were contrasting for staygreen trait also. Stay green (SG) is referred to as delayed senescence where leaves remain green even after seeds reach maturity. The data for the segregation of staygreen were recorded in the RILs of 'Chirya 3' x 'Sonalika' cross. One QTL named as *QSg.bhu-1A* was identified on the short arm of chromosome of 1A between the markers interval *Xgwm691-Xgwm752* (11.4 cM) and explained 18.9 and 9.7% of phenotypic variation in the year 2004-2005 and 2005-2006 respectively. Genetic maps of chromosome 1A were developed using 7 loci of the QTL region. The QTL was located on the short arm of chromosome 1A. The work is under way to cover the whole genome for the identification of more QTLs on other chromosomes since the QTL *QSg.bhu*-1A explained only 18.9% of phenotypic variation.

Abstract reference: ITMI2009_018

Multiplex PCR assay to detect resistance genes Lr26 and Lr37

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Leaf rust caused by *Puccinia triticina* Eriks. is one of the most prevalent diseases on the wheat in the Czech Republic. In can seriously reduce wheat yield and deteriorate the grain quality.
Breeding for resistance is the best method of plant protection. However resistant varieties carrying only one resistance gene may be overcome by new races of the pathogen and the resistance may not be durable. Pyramiding resistance genes against leaf rust can increase the resistance.
The aim of this study was to detect the presence of resistance genes *Lr26* and *Lr37* in 27 winter wheat varieties. The plants were tested in infection greenhouse test and by PCR markers. Multiplex PCR reaction with published primers was developed and optimized to detect the presence of genes *Lr26* and *Lr37* in one reaction. Gene *Lr37* was found in seven tested varieties, gene *Lr26* was found only in one tested variety and both these genes were found in three tested varieties.

Abstract reference: ITMI2009_037

Mapping QTLs for grain yellow pigment content in the cultivated durum wheat germplasm

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Grain yellow pigment content (GYP, xanthophyll pigments) is an important quality trait in durum wheat, where a bright yellow colour is a standard quality pre-requisite for semolina and pasta products. Although GYP is a quantitative trait with a relatively high heritability, a considerable portion of the cultivated germplasm still has GYP lower than the market requirements. High GYP germplasm sources have been identified and are being actively exploited in breeding programs. Thus, molecular markers tightly linked to the major GYP genes/QTLs are needed for MAS. We studied GYP in the cultivated durum wheat with a comprehensive genetic study including three RIL populations: (1) KS: Kofa (high GYP) × Svevo (medium), with 249 RILs, (2) CL: Colosseo (loow) × Lloyd (high), with 176 RILs, (3) MC: Meridiano (medium-high) × Claudio (medium-low), with 181 RILs, and a germplasm collection (Panel) of 189 cultivated durum accessions bred for Mediterranean areas plus a further panel of NorthAmerican durums. These materials were grown in Italy and in other Mediterranean countries over several years and locations and whole grain yellow index was determined as b* (Minolta b-value). Linkage maps for the three RIL populations were obtained with SSR and DArT markers from the Triticarte Durum Array v 2.0 (Maccaferri et al., 2008; Mantovani et al., 2008; Maccaferri et al., unpublished), with an average intermarker distance of 5 cM. The Panel was genotyped with 180 SSRs of known map position (as from the RIL linkage maps) and with 900 DArT markers. Major QTLs from one or two populations (average R^2 > 5% using mean data) were found in the following chr. regions: chr. 1AS (CL), chr. 2BL (KS), chr. 3BS (KS), chr. 4AL (KS), chr. 4BL (KS and CL), chr. 5A centromeric (5Ac, MC), 5BL (KS, CL, MC), chr. 6Ac (KS and CL), chr. 7Ac and 7Bc (KS and CL, homeologous positions), chr. 7AL and 7BL (KS and MC, homeologous distal position). These QTLs were validated via association mapping in the Panel and the effects of the increasing alleles were estimated. The detailed results of the QTL and association analysis will be presented and discussed.

Abstract reference: ITMI2009_038

QTL mapping for terminal heat tolerance in hexaploid wheat (T. aestivum L.)

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Post-anthesis high temperature (>30 ^{0}C) at the time of grain filling is one of the major cause of yield reduction in wheat in many environments of the world, especially in the tropical countries of the world. To identify QTLs for the traits affected by terminal heat stress, a recombinant inbred (RI) population was developed by crossing a heat tolerant hexaploid wheat (*Triticum aestivum L*) cultivar NW-1014 and heat susceptible HUW-468**.** The F_1 was advanced to F_4 and F_5 using single seed descent method in normal and off season nursery. The F_5 and F_6 generation were bulked in off season nursery to get more amount of the seed for better heat stress evaluation in two different dates of sowing under natural field conditions. The normal planting date (22nd November) was used as control while the late sown trial was planted on 5th January to expose the population to high temperature stress at grain filling. The data was recorded for grain filling duration (GFD) and thousand grain weight (TGW). Based on data of two dates, decline % GFD and decline % TGW was estimated. Significant correlation was observed between the F_5 and F_6 data for decline % GFD and decline % TGW. Out of 560 SSR markers, 186 polymorphic primers were used to genotype 148 RI lines. Two QTLs were detected on the chromosome 2B, and 7D for decline % GFD and three QTLs were detected on the chromosome 2B, 7B, and 7D for decline % TGW in F_5 and F_6 generations. The QTL on chromosome 7B was detected with the maximum LOD value of 5.83 and 10.13 for decline % TGW in F_5 and F_6 respectively in the same chromosome region. Similarly the QTLs on the chromosome 2B (LOD 1.8 F_5 and LOD 2.6 F_6) and 7D (LOD 4.5 F_5 and LOD 6.1 F_6) were detected for decline % TGW in both F_5 and F_6 generations in the same chromosome region. Another parameter, heat susceptibility index (HSI) for GFD and TGW was also used to detect the QTLs. The QTLs for HSI of GFD and TGW were detected in same region of chromosome where the QTLs for decline % of GFD and TGW were detected. The QTLs were detected in the same region for HSI (TGW) with higher LOD value of 8.28 on the chromosome 7B in F_5 as compared to 5.21 for HSI (TGW) in F_6. The threshold LOD score of 1.6 was considered to declare a QTL as significant based on the 1000 permutations. The work is under way for the fine mapping of QTLs identified in the present investigation**.**

Abstract reference: ITMI2009_039

Two major QTLs control powdery mildew resistance in durum wheat cv. Claudio

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Powdery mildew (Pm) is one of the main durum wheat fungal diseases in southern Europe. While different sources of resistance have been identified even in the elite germplasm, only a few of them have been characterized and mapped. To dissect the genetic basis of the Pm resistance carried by the cv. Claudio, a population of 181 RILs from the cross Claudio (resistant) × Meridiano (highly susceptible) has been developed and phenotypically evaluated in two replicated field trials carried out under artificially inoculated conditions during 2007 and 2008 (Bologna, Italy). A parallel mapping effort has been conducted to produce a genetic linkage maps based on 158 SSRs and 310 DArT markers (30 linkage groups). QTL analysis was carried out with a subset of evenly spaced markers. The distribution of the data collected four times during the disease developmental cycle over the two years showed a rather complex genetic control of powdery mildew response in this population. Two major QTLs controlling Pm resistance have been located on chr. 6BL (*QPm.ubo-6B*) and 7BL (*QPm.ubo-7B*) with the resistance alleles contributed by Claudio. In both years, the effect of *QPm.ubo-7B* against Pm infection declined as the disease progressed, while *QPm.ubo-6B* was undetectable in the early stage of the disease cycle and increased its effectiveness as the disease progressed. Additional minor QTLs with lower and less consistent effects across years were found on chrs. 2BS, 2BL, 6AS and 7AS, with both parents contributing the resistance alleles. *QPm.ubo-7B*, positioned in the distal end of chr. arm 7BL (*Xbarc340*, *Xgwm146* and *Xgwm344*), is possibly located in the homoeologous position of *Pm1*. The Pm resistance of Claudio is thus poligenic, with at least two major genes/QTLs with effects consistent across years and some additional minor QTLs. The pathogenicity basis of these two Pm resistance QTLs and possibly their interaction remain to be further investigated.

Abstract reference: ITMI2009_045

Rapid and Targeted Introgression of Genes into Popular Cultivars Using Marker-Assisted Background Selection

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We have optimized a marker-assisted background selection (MABS)-based gene introgression approach in wheat where 97% or more of a recurrent parent genome can be recovered in just two backcrosses (BCs). A four-step MABS method was developed based on 'Plabsim' computer simulations and wheat genome structure information. During empirical optimization of this method, double recombinants around the target gene were selected on priority in a step-wise fashion during the two BC cycles followed by selection for the non-carrier chromosomes. The average spacing among the carrier chromosome markers was <4cM. Flanking each of the 48 wheat gene-rich regions, the same number for non-carrier chromosome markers was ~12cM. Employed to introgress the *Yr15* gene into cultivar 'Zak', marker analysis of 2187 backcross plants- identified a $BC_2F_{2:3}$ plant that carried 97% of the recurrent parent genome. Phenotypically selected BC_4F_7 plants without the MABS recovered only 82% of the recurrent parent genome. Field evaluation at 17 locations showed that the MABS derived line to be either equal or superior to the recurrent parent. Based on these results, an MABS method was proposed for wheat using which any desired level of recurrent parent genome recovery is possible in just two backcrosses.

Abstract reference: ITMI2009_074

MAS for breeding varieties with low content of amylose in starch

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Low amylose content in wheat starch appears to show a positive effect on bread-making quality by improving the texture loaf volume and shelf life of bread. The use of molecular markers allows to increase efficiency of breeding strategy.

Molecular breeding has been applied for development of the new wheat genotypes with low content of amylose in the starch. The set of markers for *Wx-A1* (7AS), *Wx-B1*(4AL), *Wx-D1*(7DS) that were developed by McLauchlan et al. (2001) and Nakamura et al. (2003), were used for screening F_5 population from crossing cv. Kuyalnik x Wx-12 (99ID524). Cv. Kuyalnik is a modern Ukrainian wheat variety with prominent bread-making quality. Wx-12 (99ID524) is the line that has been provided by Dr. Graybosh (University of Nebraska, Lincoln). Wx-12 has characteristics of the absorbtion value for amylose 0,0019 at 650 nm and three null-alleles of *Wx*-genes.

We have selected 11 lines from F_5 with *Wx-A1b*, *Wx-B1b*, *Wx-D1b*. Amylose content as the measure of absorbtion for these lines were 0,03 - 0,06. The average effects of alleles *Wx-A1a*, *Wx-B1a*, *Wx-D1a* on coefficient of absorbtion were 0,050, 0,054, 0,082 units of absorbtion, respectively. There effects found twice as much high in case the only one of “wild type” allele was presented. Most probably it was contributed by known compensatory effects.

Abstract reference: ITMI2009_077

A molecular marker from TdDRF1 gene to use in wheat assisted selection aimed to improving drought tolerance.

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Crossbreeding is a fundamental tool for the genetic improvement of crops and is actively used to increase adaptability to environmental adverse conditions, such as water stress. On the other hand, traditional breeding is very time-consuming and can be largely improved by the use of new and more efficient selection criterion, allowing to associate specific genetic and/or agronomical and/or morphological traits to drought tolerance. The *Molecular Assisted Breeding* (MAB) is a modern breeding technique based on the use of genetic molecular markers as a selection tool.
The *Triticum durum* Dehydration Responsive Factor 1 (*TdDRF1*) gene codifies for transcription factors belonging to a wide multigene family, endowed of the AP2 DNA-binding domain, in durum wheat. It is a "regulatory gene", modulator of gene expression, that plays a determining role in the plant molecular response to water scarcity, inducing the expression of several downstream "functional genes" (dehydrins, etc.), which more directly contribute to the increase of the resistance/tolerance. Thus it can be considered as a good candidate to be used in MAB.
Previously, in view to identify one or more molecular markers associated to *TdDRF1* gene, we carried out a biodiversity study based on sequence polymorphisms, which were identified by collecting several *TdDRF1* genomic sequences from different wheat genotypes. Herein, we report the design of an allelic discrimination assay, to discriminate different "aplotypes" of *TdDRF1* gene, and show preliminary results on the analysis of a population of 177 durum wheat Recombinant Inbred Lines (RILs), produced by CIMMYT. IThe RILs come from MOHAWK-P1MP x COCORIT-P2MP crossbreeding: both the parents, whose "aplotypes" can be molecularly distinguished, are adapted to arid soil of Arizona and California and show high yields.

Abstract reference: ITMI2009_098

Towards the fine mapping of two major QTLs for grain yield and related morpho-physiological traits in durum wheat

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Identification of major quantitative trait loci (QTLs) for yield across a broad range of environments offers the opportunity to deploy marker-assisted selection to improve yield and yield stability of crops. In the EU-funded project IDuWUE, 249 RILs (Kofa × Svevo) were evaluated in 16 trials characterized by a broad range of water availability (rainfed and irrigated) and yield potential (from 0.5 to 5.8 t/ha). Two major epistatic QTLs on chrs. 2BL and 3BS influenced grain yield and related morpho-physiological traits, but not heading date, in a broad range of environments (Maccaferri et al., 2008, Genetics 178: 489-511). In both cases, coincidence between the QTLs for grain yield and those for plant height, peduncle length, SPAD and thousand kernel weight was observed. R^2 values calculated with the average yield values were ca. 17% for both QTLs, with a similar R^2 value due to the epistatic effect. In the FP7 EU-funded project TriticeaeGenome, near-isogenic lines for the chr. 3BS QTL are being derived in order to proceed with its fine mapping of the QTLs. In this regard, a set of 14 pairs of heterogeneous inbred families (HIFs) with the two contrasting parental haplotypes at the 3BS confidence interval have been created to obtain near isogenic lines and to better characterize the physiological basis of the QTL effects. The 3BS QTL was originally assigned to a 6 cM interval flanked by *Xgwm1034* and *Xgwm493* genomic SSRs. Additional markers have been added to this interval using sequence-derived SSRs and ISBP markers obtained from BAC end-sequences generated during the construction of the 3B physical map (Paux et al., 2008, Science, 322: 101-104). The screening of ca. 140 BES derived-SSRs allowed us to map 16 new BAC-anchored SSR markers (*Cft* series). The QTL analysis carried out with the phenotypic data generated in the IDuWUE project allowed us to better position both QTLs and to localize the chr. 3BS QTL peak in respect of the chr. 3BS physical map framework. For the QTL in the chr. 2BL region, more refined mapping is being conducted with genomic SSR markers. The QTL cluster maps in a 19 cM interval flanked by *Xwmc361* and *Xgwm1027*. Ca. 40 genomic WMS SSRs have been screened for polymorphism and 9 have been added to the local map. HIFs will be derived also for this QTL. Up to now, the two QTL regions have been marker-enriched at a resolution of ca. 1 cM.

Abstract reference: ITMI2009_125

Mapping of QTLs for yield and quality traits in Indian durum wheat

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A genetic linkage map was constructed using a RIL population ($F_{2:7}$) developed from a cross of Indian durum wheat cultivar PDW 233 and landrace Bhalegaon 4. The RILs were phenotyped across different environments and the main effect as well as epistatic QTLs for yield and quality parameters were located on the map. Test weight was influenced by three QTLs located over three chromosomes. QTL, *QTw.macs-2A,* flanked by *Xgwm71.2 – Xubc835.4* interval was the most consistent and explained up to 11.5% variation in test weight. The same QTL was also most consistent for thousand kernel weight over four environments and explained up to 15.3% variation. For grain yield, one QTL each was detected on chromosomes 2A, 4B and 6A, while two QTLs were identified on 1B. A major QTL, *QYld.macs-2A* flanked by *Xgpw2333 – Xcfa2099* explained around 15.51% variation in grain yield. A QTL for spike length, *QSl.macs-3B* detected in marker interval *Xgwm540.2 – Xgwm582.3* was consistent in three environments. For number of spikelets per spike one QTL each on chromosome 4A, 4B and 7A were identified. QTLs for kernels per spike and kernel weight per spike were located in a marker interval *Xgwm82 – Xpsp3009.3* on chromosome 6A. For gluten strength measured by sedimentation volume (SV), *QSv.macs-1B.1* flanked by *Xgwm550 - Glu-B3* was the most consistent QTL identified in all the environments. The same QTL was also associated with mixograph peak energy, peak time and total energy. The *Glu-B1* locus was at the center of another QTL responsible for SV, while, *Glu-B2* influenced SV as well as mixograph peak energy and peak time. Apart from glutenin coding loci, QTLs influencing mixing parameters and GPC were located in three other marker intervals *Xwmc48.2 – Xpsp3030* (4B), *Xgwm573 – Xbarc231.1* (7A) and *Xgwm46 – Xbarc231.4* (7B). *QGpc.macs-7B.1* was the only main effect QTL detected for grain protein content. Total 26 main effect QTLs and 10 digenic epistatic interactions (*QQ*) for gluten strength were distributed over 11 chromosomes. For yellow pigment content, five QTLs were identified on chromosome 1A, 3B, 5B, 7A and 7B across five different environments. The strongest/major QTL, *QYp.macs-7A*, located on the distal part of the long arm of chromosome 7A, explained up to 55.22% of the variation in the trait and was detected in all the five environments. The *Psy-A1* gene encoding *phytoene synthase*, a key enzyme in carotenoids biosynthesis pathway, was mapped at the centre of this QTL.

Abstract reference: ITMI2009_127

Exploring a barley fast neutron generated mutant population

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We developed a mutant population in the barley (*Hordeum vulgare* L. cv. Golden Promise) by fast neutron irradiation. To evaluate the mutant population, we screen the seeds for high free phosphate phenotypes. Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate) is the primary storage compound of phosphorous in plant seeds. Although the biosynthesis is not fully understood, several genes have been shown to be involved in the accumulation of phytic acid. To investigate the importance of different genes involved in variation of phytic acid, we are currently in the process of analysing the mutant population to locate deletions of genes involved in the biosynthesis of phytic acid using PCR based techniques.

We are also examining the natural variation in the level of phytic acid. This will be done by association studies as a part of the ERA-PG EXBARDIV project. This project includes three *Hordeum* populations. We are currently propagating 421 *Hordeum spontaneum* lines in the greenhouse to obtain seeds that can be used in this study together with barley cultivars (417 accessions) and landraces (480 accessions). The level of phytic acid in the grain will be quantified. Genotyping will be done using two Illumina Oligonucleotide Pool Assays (OPA) each containing 1536 barley SNP.

In human nutrition, phytic acid acts as an anti-nutritional factor for, in particular, Fe and Zn uptake in the digestive tract, and thus potentially contributes to the 'hidden hunger' caused by mineral malnutrition. However, phytic acid might also have anti-carcinogenic and antioxidative effects on human health. In animal husbandry, the main problem caused by phytic acid in the grain is that phosphate bound in phytic acid cannot be digested by monogastric animals. Low-phytic acid mutants might help to solve the problems that high levels of phytic acid create in food and feed consumption.

Abstract reference: ITMI2009_144

Effect of combining two genes for partial resistance to Barley yellow dwarf virus-PAV (BYDV-PAV) derived from Thinopyrum intermedium in wheat.

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The gene *Bdv2* conferring partial resistance to barley yellow dwarf virus (BYDV) was transferred to wheat chromosome 7D from *Thinopyrum intermedium*. Later, using another accession of *Th. intermedium* as a donor of resistance, a chromosome belonging to the homoeologous group 2 and conferring also partial resistance was added to the wheat genome. We report here the combining of both resistances in a genotype using GISH and a new molecular marker of *Bdv2*. Susceptible variety Sunstar, the translocation line TC14 carrying *Bdv2*, the addition line ZH with the group 2 chromosome arm carrying resistance and the line ZT with both resistances were inoculated with five previously characterized French isolates of *Barley yellow dwarf virus*-PAV exhibiting different levels of aggressivity and virulence. The resistance of TC14 and ZH were efficient against all isolates and the tests revealed an additive effect of the two sources of resistance in ZT. The resistance of the line ZT was characterized by a proportion of infected plants significantly lower than that of its parent lines TC14 and ZH and a very low virus titre.

Abstract reference: ITMI2009_159

METAPOP : When Genomics meets MARS

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Metapop is a tool build to detect, validate and use QTL in wheat.

Metapop relies on a **detection population** composed of 614 Recombinant Inbred Lines (RIL) obtained by crossing 5 elites French lines following a semi-diallel design, and on a **validation population** that allows to build Near Isogenic Lines (NIL) for any detected Quantitive Trait Loci (QTL) in only one generation. As all this material was derived from elite germplasm, QTL may be used easily in modern breeding programs. Also the best RIL could be crossed with each other to accumulate favourable alleles in an 'ideal' new genotype and therefore enrich breeding programs

Abstract reference: ITMI2009_164

SNP resources for wheat genome mapping

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A dense set of molecular markers distributed across the entire genome is critical for the development of high-density maps, genetic analysis of agronomically important traits and deployment of efficient breeding strategies in crops. Abundance in the genome and the availability of cost- and labor-effective genotyping platforms made SNPs the marker of choice for many crops. However, polyploidy and the low level of polymorphism were the major challenges for SNP discovery in wheat. Given the complexity of the wheat genome and its size, development of genome wide distributed SNPs informative for genotyping of a broad range of wheat populations will require combined efforts of many research groups. An International Wheat SNP Working Group was established to facilitate the development of SNP marker technologies by providing an organizational structure, communication and for making the information available to broad wheat community. The overview of existing genomic resources, collaborative SNP development initiatives and knowledge gaps will be presented. Availability of SNP resources to a broad international community will be discussed.

Abstract reference: ITMI2009_178

Breeding for breadmaking quality using HMW glutenin subunits in wheat (Triticum aestivum L.)

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Marker-assisted selection (MAS) hold great potential for plant breeding as it promises to expedite the time taken to produce crop varieties with desirable characters. With the use of molecular techniques it is now possible to hasten the transfer of HMW glutenin genes among varieties. Genes coding high molecular weight glutenin subunits (HMW-GS) are located at the *Glu-A1, Glu-B1*, and *Glu-D1* loci of chromosomes 1AL, 1BL, and 1DL, and are important for improving the bread-making quality of wheat. By use of protein markers we developed near isogenic wheat lines for higher sedimentation values, higher dough strength and better bread making quality. Glutenins were extracted, electrophoresed, and visualized according to standard SDS-PAGE technique for wheat. Glutenin patterns were evaluated by densitometer (Image Master DTS, Pharmacia Biotech). Homogeneity or heterogeneity, respectively, in protein composition has been studied by comparison of complete protein patterns revealed by SDS-PAGE. The high molecular weight glutenin subunits (HMW-GS) were identified according to Payne, Lawrence (1983). The genotype Kotte was used as donor for new alleles encoding HMW-GS at the locus *Glu-1B* and the Swedish bread wheat line was used as donor *21** allele at the *Glu-1A.* We have used cultivars Hana, Danubia, Elpa, Torysa, Simona, and Klea as recurrent parents. We selected desirable HMW-GS such as 5+10 (coded by *Glu-D1d)*, 21* (coded by *Glu-A1*) and 7+8 (coded by *Glu-B1*) in early generation of breeding material. The aim of the work was to use genetic markers in the segregating populations and to fix by backcross cycles the unique combination glutenin alleles and to eliminate unfavourable glutenin alleles. Our aim is development of near isogenic wheat lines for new HWM-GS pair with electrophoretic mobility between HMW-GS 6 and 9 in Hana, Simona, Torysa, Elpa and Danubia backgrounds by using of protein markers. These near isogenic lines will be valuable for future assessment of the effects of 21*, 6.2+8.3 subunits for agronomic performance and end-use quality.

Key words: marker assisted selection (MAS), wheat, *Glu* alleles.

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Session 7:
Focused groups

Abstract reference: ITMI2009_003

The International Wheat Genome Sequencing Consortium

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Bread wheat is grown on over 95% of the wheat growing area and its sequence holds the key to genetic improvements necessary to meet the increasing demands for high quality food and feed produced in an environmentally sensitive, sustainable, and profitable manner. Further, because of its recent history, hexaploid wheat is a very good model to study polyploidy, a driving force for plant genome evolution. The International Wheat Genome Sequencing Consortium (IWGSC) was established by plant scientists, breeders, and growers who are dedicated to sequencing the wheat genome to enhance our knowledge of its structure and function as well as deploy state-of-the-art molecular tools to accelerate wheat improvement and meet the challenges of the 21st century. The Consortium is committed to ensuring that the wheat genome sequence and the resulting DNA-based tools are available for all to use. To achieve the vision of a sequenced wheat genome, the IWGSC develops strategic plans with short- and mid-term goals, defines areas of coordination, facilitates and coordinates research projects and funding efforts at the national and international levels, develops and supports the design of research proposals, provides a framework for the establishment of common guidelines, protocols, and resources, and organizes scientific meetings and workshops. The IWGSC is governed by six co-chairs, a Coordinating Committee, and an executive director. General membership is open to any individual, laboratory, or entity with an active interest in meeting IWGSC objectives. The mission, goals, organizational structure, projects, and online membership registration are available at http://www.wheatgenome.org.

Abstract reference: ITMI2009_161

Action FA0604: Triticeae Genomics for the Advancement of Essential European Crops

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Europe faces the challenge of delivering safe, high-quality, and health-promoting food and feed as well as bio-products in an economical, environmentally sensitive, and sustainable manner across environments that face climatic change and increasing abiotic and biotic stresses. Triticeae cereals (wheat, barley and rye) are essential in human and domestic animal nutrition and are arguably the most important crops for European agriculture. Existing germplasm resources and current breeding methods alone are insufficient for understanding the mechanisms underlying important traits and for catalysing a quantum leap in yield, sustainability and quality improvement. Major advances in crops will require a broad suite of direct genomics approaches, built on relevant data from model plants (rice, Brachypodium). Such a strategy is massively complex and can only be carried out efficiently at the international level. The COST Action will coordinate, focus and strengthen national and pan-European Triticeae genomics to improve sustainability and value of the crops. For more information on this COST Action programme see http//tritigen.ari.gov.cy/

Abstract reference: ITMI2009_163

The International Barley Sequencing Consortium (IBSC)

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The International Barley Sequencing Consortium was established in 2006 as a formal network for joining forces and coordinating international efforts towards the establishment of a reference sequence of the 5 Gbp barley (*Hordeum vulgare* L.) genome. Eight institutions from six countries committed to synchronize their activities according to an agreed roadmap to sequence the barley genome (http://barleygenome.org, Schulte et al. 2009, Plant. Physiol. 149:142-147). Due to the large size and the immense repetitive DNA content of the barley genome a rather conservative approach was prioritized relying on the physical mapping of the barley genome, high-density anchoring of the physical to the genetic map and the utilizing such resource as a template for the generation of a high quality reference genome sequence following a map-based clone-by-clone sequencing strategy. This outline is substantiated by a number of complementary attempts of pilot sequencing (i.e. fl-cDNA, selected BAC clones, reduced representation libraries, barley genomic shotgun, BAC end sequences) to gather additional information about the barley genome structure which might impact and maybe even lead to a revision of the original strategy. This is of crucial importance in the context of the recent availability of high-throughput next generation sequencing platforms. The presentation will review the current status of IBSC activities towards sequencing the barley genome.

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Céréales Vallée, the Competitiveness Cluster in "Innovation in cereals"

Céréales Vallée has two main objectives:

International - To design plants for the future, the vital foundations of competitiveness in agriculture and cereal chains worldwide, by increasing and developing genetic resources using plant biotechnologies.

National and regional - Valorizing agricultural production through efficient agro-industrial chains, underpinning socio-territorial development, by analyzing and improving the composition of plants in order to provide a better response to new food and non-food needs.

Céréales Vallée has created a skills platform in Auvergne that is unique and recognized internationally for its innovation in cereals, the raw materials central to global food, non-food and environmental challenges that are crucial to our society.

The Cluster brings together almost 500 players in the cereal sector, involved in research, industry, services and training. Strengthening the sustainable nature of value chains is at the heart of the Céréales Vallée strategy, which is based around 3 themes:

- Cereals for the future: produce more and better for sustainable agriculture
- Cereals for health and nutrition: satisfy the needs of human and animal food production
- Non-food cereals: contribute to developing sustainable biomaterials.

As part of this strategy, Céréales Vallée has developed partnerships with French and international players. The Cluster assists in setting up and coordinating innovative research and development, industrial, educational and international projects.

Since 2005, Céréales Vallée has provided a strong dynamic response for its members to inspire their innovation within a context of strong international competitiveness.

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Born on the lands of Auvergne, Limagrain is an international agricultural co-operative group, specialized in seeds and cereal products.

It is the 4th largest seeds company in the world with its subsidiary Vilmorin & Cie, the European leader in functional flour with Limagrain Céréales Ingrédients and the 2nd largest industrial baker in France with Jacquet.

The Group makes annual sales of over one billion euros and has a headcount of nearly 6000, including 1200 researchers, spread over 36 different countries.

Limagrain is pursuing its mission through a balanced vision of the surrounding world: a difference it makes a point of cultivating.

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BIOGEMMA, a leading European Research Company in Plant Biotechnology, developed and funded by the farming community, brings together specialists in plant biotechnology and plant breeding, and representatives of major French actors in seed activities: the cooperative groups LIMAGRAIN and EURALIS, the company RAGT, as well as the grain and oil and protein sectors represented by UNIGRAINS and SOFIPROTEOL.

Through its activities, BIOGEMMA contributes to the creation of new seeds responding to the great challenges of agriculture:
For corn, wheat, sunflower or oilseed rape, its 65 researchers describe, explore and enrich the genetic diversity of species cultivated in Europe, in a process of responsibility and controlled progress.

Its four key issues are:

- Participate in the advancement of knowledge through analysis of plant genomes;
- Contribute to a better environment by developing plants resistant to pathogens, insect pests, plants that use nitrogen more efficiently or consume less water;
- Describe and enhance the biodiversity of plant species through new ways to improve plant increasing the genetic base of cultivated species;
- processing quality.

BIOGEMMA takes part to many partnerships that involve academic labs, institutions and private companies, locally and worldwide. BIOGEMMA is still willing to initiate new collaborations with partners that share common visions.

BIOGEMMA

Date of the partnership: 1997
Area: European Research Company in Plant Biotechnology for crop species
Shareholders: Vilmorin & Cie * (55%), Euralis (16%), RAGT (10%), Sofiprotéol (9.5%), Unigrains (9.5%)

Works: genomics, post-genomics and transgenesis
Permanent staff: 83 people
Laboratories: Clermont-Ferrand (63), Mondonville (31)
CEO: Pascual Perez
* includes the seed activities of Limagrain

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RAGT, Rouergue Auvergne Gévaudan Tarnais

Named after the 4 regions in France where the company has its origins, RAGT is strongly rooted in Agriculture. It maintains its aim of being ambitious, innovative and competitive within this field.

From Aveyron to Europe: Founded by Aveyronnais farmers whose families are still its shareholders, RAGT's success has resulted from its ability to maintain a local approach to the business while developing on an international scale. RAGT is now established in all the major agricultural areas of Europe.

RAGT at the heart of agriculture: RAGT is made up of 2 core businesses:

- Seed Business: RAGT develops, produces and markets seed all over the world. It is active in all the major crop species used in European Agriculture.
- Agricultural Supply Business: RAGT Plateau Central supplies advice and agricultural products to over 10,000 farms in the Averyon area of France.

RAGT - innovative research: Over 200 new varieties registered each year from 22 different crop species including all Triticeae species. RAGT invests over 12% of its turnover in research and development which involve 190 breeders and technicians, 15 research stations across Europe and 4 laboratories as well as partnerships with public sector labs for the development of dense genetic maps based upon SNP markers. RAGT also invests a lot in cereal genomics with two biotechnology laboratories dedicated to use genomic tools to associate agriculturally important genes to molecular markers (typically SNPs or SSRs) and to apply these latter across breeding programmes (marker assisted selection, MAS).

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