The complex regulation of wheat grain storage protein synthesis unraveled through GWAS

Catherine Ravel, Anne A. Plessis, Jacques J. Bordes, Nathalie N. Duchateau, Rémi Boussicut, François Balfourier, Pierre Martre

Cereals for Food, Feed and Fuel – Challenge for Global Improvement

BOOK OF ABSTRACTS

Ulrike Lohwasser & Andreas Börner
(eds.)

Wernigerode 2014

EUCARPIA Cereals Section –
I T M I Joint Conference
Wernigerode, Germany, June 29 – July 4, 2014

Cereals for Food, Feed and Fuel – Challenge for Global Improvement

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Program

June 29, 2014

12:00 - 21:00	Registration	

Session – IWGSC Workshop

13:00-13:20	Gabriel Keeble-Gagnere	Sequence-based assembly of wheat chromosome 7A and comparison of its features to 7A from diploid progenitors
13:20-13:40	Patrizia Galeffi	From gene to genomics: the *DRF1* gene sequence of bread wheat chromosome 7B based on individual MTP BAC sequencing using pair end and mate pair libraries
13:40-14:00	Vijay Tiwari	Radiation Hybrid Mapping: High resolution RH maps of D-genome chromosomes of hexaploid wheat
14:00-14:20	Jan Bartoš	Physical map of the wheat chromosome arm 3DS
14:20-14:40	Martin Mascher	Genetic anchoring of the chromosome shotgun assembly of bread wheat by population sequencing
14:40-15:00	Ana Luísa Garcia-Oliveira	Molecular characterisation of *TaMATE1* homoeologues genes and the analysis of organic acids genes expression under Al stress in bread wheat
15:00-15:30	Coffee Break	
15:30-15:50	Aurelie Evrard	Epigenomic maps of the bread wheat chromosome 3B

15:50-16:10	Hana Simkova	BioNano genome map of wheat chromosome arm 7DS supports accurate sequence assembly
16:10-16:30	Giampiero Valè	An integrated approach for the physical mapping of wheat chromosome 5A
16:30-16:50	James Cockram	MAGIC wheat: Multi-parent populations for the genetic dissection of agronomic traits
16:50-17:10	Naser Poursarebani	The Whole Genome Profiling™ for physical mapping of wheat chromosome 6A
17:10-17:30	Odd-Arne Olsen	A draft sequence of bread wheat chromosome 7B based on individual MTP BAC sequencing using pair end and mate pair libraries
19:00 - 22:00	Welcome Reception	

June 30, 2014

07:30 - 18:00 Registration

Session I – Genetic Resources

08:15 - 08:30	Andreas Börner / Zoltán Bedő	Opening Remarks
08.30 - 09:15	Peter Wenzl (invited speaker)	Seeds of Discovery (SeeD): A project to systematically characterize and mobilize novel genetic variation from genebanks into breeding programs
09:15 - 09:45	Luzie U. Wingen	Unravelling of genetic diversity and domestication trends for allele discovery in a bread wheat landrace collection

09:45 - 10:15	Manuela Nagel	Seed conservation in *ex situ* genebanks – genetic and biochemical markers of viability illuminate mechanisms of seed longevity in barley
10:15 - 10:45	Coffee Break	
10:45 - 11:00	Christoph Dockter	Quantitative variation of barley culm length and sturdiness is controlled by *BREVIARISTATUM, BRACHYTIC* and *ERECTOIDES* gene loci involved in brassinosteroid hormone metabolism
11:00 - 11:15	Andreas Maurer	Allele mining in wild barley: Finding new exotic genes which control flowering time in the barley nested association mapping (NAM) population HEB-25
11:15 - 11:30	Rajiv Sharma	Genetic basis of photoperiod-insensitivity in barley
11:30 - 11:45	Joanne Russell	Diversity in heritage barleys: Adaptation on a local scale
11:45 - 12:00	Isabelle Colas	The barley meiotic tool box: Fundamental science for practical breeding
12:00 - 12:15	Simon Orford	Germplasm development in the landrace "Pillar" of the Wheat Improvement Strategic Programme
12:15 - 12:30	Marta Molnar-Lang	Evaluation of flowering time, β-glucan content and tillering of wheat/barley introgression lines
12:30 - 14:00	Lunch	

Session II – Grain Yield

14:00 - 14:45	Zoltán Bedő (invited speaker)	Looking for new engines of yield improvement
14:45 - 15:15	Jochen Reif	Hybrid breeding in wheat

15:15 - 15:45	Wolfgang Friedt	Breeding progress and trends of winter wheat yield in Germany
15:45 - 16:15	Coffee Break	
16:15 - 16:30	Thorsten Schnurbusch	Genetic analysis of pre-flowering development in barley (*Hordeum vulgare* L.)
16:30 - 16:45	Hazel Joane Bull	*VRS3*: A fourth piece in the barley row type puzzle
16:45 - 17:00	Ilka Braumann	Cloning and characterization of a semi dwarfing gene in barley
17:00 - 17:15	Sara Milner	A multiparental cross population for mapping of QTL for agronomic traits in durum wheat (*Triticum durum* Desf.)
17:15 - 17:30	Julien Pierre Bonneau	Towards positional cloning of a grain yield QTL on chromosome 3B in wheat (*Triticum aestivum* L.)
17:30 - 17:45	Van Giang Tran	BWGS: A Breed Wheat Genomic Selection pipeline
17:45 - 18:00	Esther Mitterbauer	Analyses of the response of 100 different barley genotypes to future CO_2 concentrations in order to optimize the CO_2 fertilization effect
18:30 - 20:00	Dinner	
20:00 - 22:00	Poster Session	
20:00 - 22:00	IWGSC Committee Meeting	

July 01, 2014

Session III – Abiotic Stress Resistance

08.30 - 09:15	Silvio Salvi (invited speaker)	Leveraging genomics approaches to enhance abiotic stress resistance in cereals
09:15 - 09:45	Wiltrud Renate Erath	Identification of genomic regions involved in frost tolerance in winter rye
09:45 - 10:15	Kerstin Neumann	Growth, wilting and recovery – digital imaging and modelling of vegetative biomass growth in seasonal drought conditions
10:15 - 10:45	Coffee Break / Group Foto	
10:45 - 11:00	Delphine Fleury	Genetic control of plant growth response of wheat to drought
11:00 - 11:15	Bruno Bouffier	Environmental chacterization and QTL detection to dissect wheat tolerance to drought and high temperature
11:15 - 11:30	Hamid Shirdelmoghanloo	Genetic analysis of heat tolerance components in wheat
11:30 - 11:45	Benedict Chijioke Oyiga	Genetic analysis of tolerance to salt stress in wheat using association mapping
11:45 - 12:00	Youko Oono	Genome-wide transcriptome analysis of Pi-stressed wheat seedlings
12:00 - 12:15	Markus Kuhlmann	Tiller number in barley is influenced by ABA
12:15 - 12:30	Mohammad Sameri	Identification of favorable alleles in wild barley for waterlogging tolerance
12:30 - 14:00	Lunch	

Session IV – Biotic Stress Resistance

14:00 - 14:45	Frank Ordon (invited speaker)	Harnessing resistance to biotic stress in cereals – past and future
14:45 - 15:15	Beat Keller	Suppression among alleles of an NB-LRR resistance gene interferes with resistance in F_1 hybrids and allele-pyramided wheat plants
15:15 - 15:45	Åsmund Bjørnstad	*Fusarium* head blight of oats: Infection, germination and resistance
15:45 - 16:15	Coffee Break	
16:15 - 16:30	Mehmet Cakir	Genomics-assisted cereal pre-breeding for pest and diseases for global food and bio-security
16:30 - 16:45	Marion Röder	Association mapping in European winter wheat
16:45 - 17:00	Marco Maccaferri	Genome-wide association scan for stripe rust (*Puccinia striiformis* f. sp. *tritici*) response in spring hexaploid wheat (*Triticum aestivum* L.) from worldwide
17:00 - 17:15	Severine Hurni	Powdery mildew resistance gene *Pm8* derived from rye is suppressed by its wheat ortholog *Pm3*
17:15 - 17:30	Ana Maria Castro	Screening for *Sipha maydis* resistance in Argentinean commercial varieties
17:30 - 17:45	Hanan Sela	Linkage disequilibrium and association analysis of stripe rust resistance in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) population in Israel
17:45 - 18:00	Dragan Perovic	Towards deciphering a novel barley yellow dwarf virus (BYDV) tolerance introgressed from *Hordeum bulbosum* by the use of genomic resources and Next-Generation-Sequencing (RNASeq and exome capture)

18:30 - 20:00	Dinner	
20:00 - 22:00	Poster Session	
20:00 - 21:00	Eucarpia Member Meeting	

July 02, 2014

Session V – Quality for Food and Industrial Use

08.30 - 09:15	Heinrich Grausgruber (invited speaker)	Cereal quality – time for a paradigm shift?
09:15 - 09:45	Christoph Ulrich Germeier	Quality traits relevant for human nutrition in European oat genetic resources collections
09:45 - 10:15	Elena Khlestkina	Purple-grained wheat: Genetic control, molecular mechanisms and practical value
10:15 - 10:45	Coffee Break	
10:45 - 11:00	Catherine Ravel	The complex regulation of wheat grain storage protein synthesis unraveled through GWAS
11:00 - 11:15	Domenico Lafiandra	A TILLING strategy to improve the nutritional health value of wheat
11:15 - 11:30	Archana G. Patil	Identification of functional mutations in arabinoxylan synthetic genes in hexaploid wheat
11:30 - 11:45	Sebastian Förster	Screening for speltoid off-types in bread wheat – Discrimination of alleles and copy numbers at the domestication gene Q using quantitative pyrosequencing

11:45 - 12:00	Lajos Bona	End-use traits of *Triticale*: Tasks for human utilization
12:30	Excursion to Breeders	
	Alternative Options:	
	RAGT Silstedt	
13:30	Lunch	
14:30	Visit Fields	
16:30	Bus to Gatersleben	
	Strube Söllingen	
14:00	Lunch	
15:00	Visit Fields	
16:30	Bus to Gatersleben	
	SW Seed Hadmersleben	
14:00	Lunch	
15:00	Visit Fields	
16:30	Bus to Gatersleben	
17:00	Barbecue IPK Gatersleben (with visits of the genebank, field, greenhouse, and automatic phenotyping systems)	
22:00/23:00	Transfer to Wernigerode	

July 03, 2014

Session VI – Mapping, Cloning and Beyond

08.30 - 09:15	Takao Komatsuda (invited speaker)	Domestication of barley: Independent mutations in *Btr1* and *Btr2* genes generated a tough rachis that facilitated harvesting of grain
09:15 - 09:45	Luke Douglas Ramsay	Effect of temperature on recombination in barley
09:45 - 10:15	Etienne Paux	A reference sequence of wheat chromosome 3B reveals structural and functional compartmentalization
10:15 - 10:45	Coffee Break	
10:45 - 11:30	Evans Lagudah (invited speaker)	Broad spectrum resistance in wheat: Retrospect and prospects
11:30 - 11:45	Nicolai Adamski	Natural variation at the *Inhibitor of wax 1* (*Iw1*) locus has led to the formation of different haplotypes for glaucousness
11:45 - 12:00	Miroslav Valárik	Construction of physical map for the locus introgressed to bread wheat from *Triticum militinae* conferring powdery mildew resistance
12:00 - 12:15	Carlos P. Cantalapiedra	BARLEYMAP: Unlocking sequence-enriched resources for breeders
12:15 - 12:30	Abraham Korol	A fast algorithm and software for building ultra-dense genetic maps in the presence of genotyping errors and missing data
12:30 - 14:00	Lunch	

14:00 - 14:15	Katie Baker	The epigenome of barley: Global profiling of histone modifications in barley chromatin using ChIP-seq
14:15 - 14:30	Bernd Hackauf	Genome-wide association mapping reveals novel insights in the genetic architecture of agronomic and quality traits in rye hybrids
14:30 - 14:45	Martin Mascher	Mapping-by-sequencing accelerates forward genetics in barley
14:45 - 15:00	Thomas Wicker	Analysis of highly repetitive BAC clones reveals rapid evolution of super-variable gene clusters in Triticeae

15:30	Departure to Wernigerode Train Station
16:00	Departure Train to Magic Event
00:30	Arrival Wernigerode

July 04, 2014

Session VII – Future Challenges and Innovations

08.30 - 09:15	Andreas Graner (invited speaker)	Innovations and challenges in crop genomics
09:15 - 09:45	Andrecj Kilian	Integration of DArTseq genome profiling and KDDart IT platform into breeding of Triticeae crops
09:45 - 10:15	Gilles Charmet	Genomic selection in wheat: Accuracy in cross-population prediction
10:15 - 10:30	Mark Earl Sorrells	Genomic Selection: Training populations and GxE
10:30 - 10:45	Kellye Eversole	The IWGSC: Building the sequence-based foundation for accelerated wheat breeding

10:45 - 11:00	Martin Ganal	Large scale SNP genotyping with optimized marker sets for cost-efficient wheat breeding using molecular markers
11:00 - 11:30	Coffee Break	
11:30 - 11:45	Jochen Kumlehn	Efficient and heritable genome engineering in barley using customized TALE-nucleases
11:45 - 12:00	Dionysia A. Fasoula	Sustainable agricultural production in Cyprus and the crucial role of density-independent varieties under global climate change
12:00 - 12:15	Davide Bulgarelli	Structure and evolution of the microbiota thriving at the barley root-soil interface
12:15 - 12:30	Angela Maria Bernal Vasquez	Comparison of different spatial models for field trials in stage-wise analysis for genomic selection in rye (*Secale cereale* L.)

Honorary Session

12:30 - 13:00	Thomas Miedaner	Improvement of hybrid rye for yield traits and drought tolerance by genomics-based breeding
13:00 - 13:30	Manfred Schönleben	Genomic selection in hybrid rye breeding
13:30 - 14:00	Andreas Börner	Closing Remarks

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IWGSC-Workshop

Congratulations

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IWGSC Early Career Awardees

Katherine Jordan
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Naser Poursarebani
EUCARPIA-ITMI

Vijay Kumar Tiwari
EUCARPIA-ITMI

Sequence-based assembly of wheat chromosome 7A and comparison of its features to 7A from diploid progenitors

G Keeble-Gagnere[1], J Nystrom-Persson[1], C Cavanagh[2], D Fleury[3], H Webster[1], R Appels[1]

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[2] *CSIRO Plant Industry, Clunies Ross Street, Back Mountain ACT 2601*
[3] *Australian Centre for Plant Functional Genomics, Hartley Grove, Urrbrae, Glen Osmond SA 5064*

This presentation provides a report on the sequencing the genome of chromosome 7A from wheat. The sequencing of chromosome 7A project (GRDC/BioPlatforms Australia-funded) has established the physical assembly of BAC clones prepared from flow sorted ditelocentric chromosomes. The Illumina Hiseq sequencing of BAC pools (AGRF) comprise physical contigs of 1-2 Mb genomic DNA which were assembled using the SNAPshot DNA fingerprinting of individual BACs with 5 restriction endonucleases. Extensive anchoring of the sequence assemblies was achieved initially using the 9K and 90K SNP chip-based molecular genetic maps and currently using the high-resolution map of chromosome 7A in the MAGIC population.

Although the assembly still needs refinement through additional sequencing of long insert mate pair libraries, comparative analyses have shown that the assembly is robust and capable of providing new insights into agronomically important loci. Some specific examples will be discussed which utilize an extensive in-house RNA-seq transcriptome for characterizing the genes in regions of interest.

From gene to genomics: The *DRF1* gene sequence of bread wheat chromosome 7B based on individual MTP BAC sequencing using pair end and mate pair libraries

K Thiyagarajan[1], A Latini[1], D Di Bianco[1,2], C Cantale[1], K Ammar[2], P Galeffi[1]

[1] *ENEA CR Casaccia, Rome, Italy*
[2] *CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo), México*

The dehydration responsive element binding (DREB) proteins are important transcription factors that contribute to stress endurance in plants triggering the expression of a set of abiotic stress-related genes.

The *Dehydration Responsive Factor 1* (*DRF1*) gene belongs to DREB-family and is involved in the abiotic stress response in *Triticeae*. The gene was firstly isolated in barley and durum wheat and successively homologous genes were isolated/identified in other plants. It consists of four exons and three introns and expresses through an alternative splicing mechanism and possibly evolved by the insertion of a non-autonomous transposon followed by exonization.

The genetic diversity of *DRF1* gene was analysed in *Triticeae* and in other plants looking for relevant features such as SNPs, SSRs, gaining knowledge about the intra- and inter-specific variability. Based on the gene variability, a PCR test resulted able to selectively discriminate a durum RIL population in significant correlation with grain yield under water-limited conditions.

By using information in IWGSC database, the gene was mapped at Chromosome 1 in all three genomes, A, B and D, and its promoter was quickly isolated and sequenced. The achieved information was used to build specific inducible expression vectors.

The expression profile of the *DRF1* gene was studied analysing the modulation of its three transcripts during dehydration experiments in greenhouse and in open controlled fields. Differences were observed in molecular patterns, thus, suggesting a genotype dependency of gene expression in response to the stress induced. The structural effect of genetic variability was evaluated and related to the variable expression in the different genotypes.

Radiation Hybrid Mapping: High resolution RH maps of D-genome chromosomes of hexaploid wheat

V K Tiwari[1,5], O Riera-Lizarazu[1], A Kumar[2], H Gunn[1], A Denton[2,4], Y Wang[4], T Drader[4], F Ghavami[2], J Hegstad[2], M Gitt[4], Y Q Gu[4], M-C Luo[3], G Lazo[4], B S Gill[5], S F Kianian[2], J M Leonard[1]

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[2] *Department of Plant Sciences, North Dakota State University, Fargo, ND 58105*
[3] *Department of Plant Sciences, University of California, Davis, CA 95616*
[4] *USDA-ARS, Western Regional Research Center, Albany, CA 94710*
[5] *Wheat Genetics Resource Center, Department of Plant Pathology, Kansas State University, Manhattan, KS, 66506*

Radiation Hybrid (RH) mapping is an efficient physical mapping method as it does not rely on meiotic recombination. It offers a unique opportunity to solve the recombination and map resolution based issues of genetic mapping, for developing high resolution physical maps of wheat chromosomes. To construct RH based high resolution maps of D- genome chromosomes of hexaploid wheat 'Chinese Spring' we developed three independent RH panels: seed irradiation based panel (~2000 RHs), pollen plant panel (~600 RHs) and endosperm panel (1000 RHs). Generated RH panels were tested for low marker retention (25%-50%) and breaks in multiple chromosomes using a set of molecular markers. Average marker retention frequencies of the selected seed irradiation, pollen plant and endosperm panel were 3.1%, 28.5% and 45.8%, respectively. Pollen plant panel and endosperm panel showed breaks in multiple chromosomes, whereas seed panel showed breaks in a few chromosomes only. Set of highly informative lines including pollen plant panel (188) and endosperm panels (94) were selected and used for high throughput genotyping. Ninety-four seed panel lines were also included in the study for the sake of comparison among different panels. We developed a high throughput genotyping system based on NimbleGen array and a total of 37,230 markers (6,330 gene markers + 30,900 repeat junction markers) were included in the final genotyping array. Repeat junction markers were designed from Roche-454 genome sequences of the wheat D-genome progenitor, *Aegilops tauschii.* These markers were tested and validated on nullisomic and tetrasomic lines of all seven D-genome chromosomes of Chinese Spring. Additionally these markers were mapped on deletion stocks of D-genome chromosomes. Genotyping data returned us ~3,500 markers per chromosome. Analysis of data suggested that an average map resolution of ~450 kb can be achieved using this approach. Since in the present study we focused on RH mapping of D- genome chromosomes of 'Chinese Spring' it allowed us to compare our results with *Aegilops tauschii* physical maps. Detailed analysis on construction of radiation induced physical maps, its application in anchoring of BAC contigs and comparative analysis between Chinese Spring and *Ae. tauschii* D-genome maps will be presented.

Physical map of the wheat chromosome arm 3DS

J Bartoš[1], K Cviková[1], J Šafář[1], H Šimková[1], M Alaux[2], H Bergès[3], F Cattonaro[4], N Stein[5], K Mayer[6], J Doležel[1]

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[3] *Centre National de Ressources Genomiques Vegetales, Chemin de Borde Rouge 24, 31326 Castanet Tolosan, France*
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[6] *Institute for Bioinformatics and Systems Biology, Helmholtz Center f. Health and Environment, Ingolstadter Landstrasse 1, D-85764 Neuherberg, Germany*

Creation of a physical map is one of the important steps towards sequencing complex genomes. For bread wheat (*Triticum aestivum L.*), construction of BAC libraries from individual chromosomes and chromosome arms purified by flow-sorting is a generally accepted strategy. As the next step, High-Information Content Fingerprinting (HICF) is widely used in plant genomics for physical mapping. Here we present a physical map of the short arm of wheat chromosome 3D (3DS) with the estimated size of 321 Mbp. The map consists of 657 contigs assembled using Finger Printed Contigs (FPC) and Linear Topological Contig (LTC) algorithms. BAC contigs of the physical map were anchored to a gene set, which was virtually organized along the chromosome arm based on colinearity to gene order in genomes of sequenced relatives of wheat (GenomeZipper) and a genetic map constructed using F7 mapping population Chinese Spring x Renan. Shotgun sequences generated by the International Wheat Genome Sequencing Consortium were integrated to the physical map to improve its utility for positional gene cloning. To date, thousands of sequences were positioned in the physical map allowing easy access to most of the genes on 3DS chromosome arm.

This work was supported by Czech Science Foundation (grant 13-8786S) and IGA PrF/2012/001.

Genetic anchoring of the chromosome shotgun assembly of bread wheat by population sequencing

M Mascher[1], J Chapman[2], J A Poland[3], M Pfeifer[4], U Scholz[1], K Barry[2], International Wheat Genome Sequencing Consortium (IWGSC)[7], K Mayer[4], R Waugh[5], D S Rokhsar[2], G J Muehlbauer[6], N Stein[1]

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[4] *MIPS/IBIS, Helmholtz Zentrum Munich, Ingolstädter Landstr. 1, 85764 Neuherberg*
[5] *The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK*
[6] *University of Minnesota, Department of Agronomy and Plant Genetics, St. Paul*
[7] *International Wheat Genome Sequencing Consortium*

Bread wheat (*Triticum aestivum* L.) is an allohexaploid species with a highly repetitive 17 Gb genome. Its sheer size and complexity have made sequencing the wheat genome a challenging task. We have recently developed the POPSEQ method to genetically anchor and order shotgun assemblies of large and complex genomes with sequencing data from segregating populations. POPSEQ has been used successfully to anchor the whole-genome shotgun assembly of barley and has been validated by comparison with the integrated physical and genetic map of barley. Here, we report about POPSEQ in wheat. We sequenced the two parents and 90 doubled haploid lines of the Synthetic W7984 x Opata M85 population to ~1x whole genome coverage on the Illumina Hiseq2000. Sequence reads were aligned against the sorted chromosome assemblies of cultivar Chinese Spring. Computational variant detection yielded 17.2 million single nucleotide polymorphisms, of which 13.2 million were placed onto a genetic framework map constructed through genotyping-by-sequencing. Using this high-density marker dataset, 4.4 Gb of the 10.1 Gb chromosome shotgun sequence assembly were assigned to genetic positions. POPSEQ chromosome assignments were in excellent agreement with flow-sorting based chromosome assignments and were largely collinear with the synteny-based gene order constructed with the GenomeZipper method. Combining the results of POPSEQ and the GenomeZipper, more than 75,000 high-confidence gene models could be positioned on the 21 chromosome of bread wheat. These promising results obtained in a small doubled haploid population, hold the promise of more comprehensive anchoring with higher resolution when additional populations will be sequenced. POPSEQ data will also be instrumental in anchoring physical maps and BAC-based sequences of individual wheat chromosomes.

Molecular characterisation of *TaMATE1* homoeologues genes and the analysis of organic acids genes expression under Al stress in bread wheat

A L Garcia-Oliveira[1,2], P Martins-Lopes[1], R Tolrá[3], C Poschenreider[3], H Guedes-Pinto[1], C Benito[2]

[1] *CGB/IBB, UTAD, Portugal*
[2] *UCM, Spain*
[3] *UAB, Spain*

The efflux of organic anions from roots is one of the major mechanisms for Al-detoxification in plant species. In past, ALMT1 associated with Al-activated efflux of malate has been extensively studied in bread wheat. Recently, the importance of citrate efflux for Al-tolerance has also been reported in cereals. Bread wheat comprised from three genomes (AABBDD) and most of genes can be found in triplicates with one copy on each genome. The natural hybridity as well as large genome size with highly repetitive DNA sequences makes it a difficult crop for genomic research such as cloning of homoeologues genes that represents a major challenging task to improve this cereal. . Therefore, we aimed to clone a citrate transporter gene, and further investigate the upstream variations in organic acids transporter genes in diverse genotypes of bread wheat.

Aneuploid stocks of Chinese-spring revealed that *TaMATE1* genes are located on the long arm of homoeologous group 4 chromosomes. Real-time-qPCR detected the preponderance of *TaMATE1-4B* followed by *TaMATE1-4D*, whereas *TaMATE1-4A* seemed to be silence. *TaMATE1* transcript levels were observed quite stable under Al-stress. Noticeably, higher levels of *TaMATE1*, particularly homoeologue *TaMATE1-4B*, and *TaALMT1* transcripts were detected in the root apices than shoots of bread wheat. In contrast to Al-sensitive genotype Anahuac, Barbela7/72/92 presented a very high basal levels of *TaALMT1* and *TaMATE1* transcripts in roots and shoots tissues. Interestingly, Al-tolerant genotype also presented a Sukkula-like transposon (SLT) and tandem repeats in the promoters of *TaMATE1-4B* and *TaALMT1*, respectively.

TaMATE1 belongs to multi-drug transporter protein family whose high basal transcripts level in Al-tolerant genotype and quite stable expression under Al-stress conditions in both Al-tolerant and sensitive genotypes suggest that *TaMATE1* is associated with the constitutive efflux of citrate in bread wheat. Finally, the presence of SLT and tandem repeats also showed a strong correlation with Al-tolerance in hexaploid wheat cultivar Barbela 7/72/92.

Epigenomic maps of the bread wheat chromosome 3B

A Evrard, E Paux

Institut National de la Recherche Agronomique, Université Blaise Pascal, Unité Mixte de Recherche 1095 GeneticsDiversity and Ecophysiology of Cereals, F-63100 Clermont-Ferrand, France

Eukaryotic genomes are organized in chromatin, which not only packages the DNA, but also functions as a carrier of epigenetic information, including DNA methylation, nucleosome positioning, histone variants and their post-translational modifications. Together these epigenetic marks impact many cellular processes operating on DNA such as transcription, replication, recombination and repair. They are further essential players in the vital control of potentially deleterious DNA sequences such as transposable elements. In large plant genomes, like wheat, where genes are interspersed in considerable amounts of transposable elements that can make up to 80% of the genome, epigenetic marks are likely to play a pivotal role in the regulation of gene expression and maintenance of genome integrity.

In wheat, genome-wide (or chromosome-wide) epigenetic studies have long been hampered by the lack of a reference sequence. However, using a chromosome-based approach, the INRA GDEC recently produced the first reference sequence of a wheat chromosome: 3B.

Taking advantage of this unique genomic resource, we initiated a project aiming at exploring the epigenetic landscape along the bread wheat chromosome 3B in order to better understand the two main chromatin structures: euchromatin and heterochromatin in a polyploid and highly repetitive genome.

The strategy relies on a combination of immunoprecipitation targeting either methylated DNA (MeDIP) or chemically modified histones (ChIP) with sequence capture to focus on the chromosome 3B gene space. DNA fragments are then sequenced on an Illumina HiSeq2000 and reads are aligned to the chromosome 3B genes in order to explore the localization of each mark at each genetic loci and at the chromosome level. The combination of these epigenetic marks will allow us to define chromatin states along the bread wheat chromosome 3B and investigate the distribution patterns of these marks according the gene density or particular motifs of the gene space.

BioNano genome map of wheat chromosome arm 7DS supports accurate sequence assembly

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Specific features of the bread wheat genome, such as its size (17 Gbp), polyploid nature and prevalence of repetitive sequences make whole-genome-sequencing a challenge. BAC-by-BAC sequencing based on chromosomal physical maps, which has been adopted by the International Wheat Genome Sequencing Consortium as the key strategy, reduces problems due to the complexity and polyploidy. However, high content of repeats (over 80%) still prevents unambiguous sequence assembly, both on a small (BAC clones) as well as large (whole chromosomes) scale. Availability of a high-resolution genomic map that would reveal discrepancies in sequence assemblies and provide a clue for sequence scaffolding would be highly beneficial for obtaining an accurate and complete genome sequence in wheat.

Aiming to produce a quality reference genome sequence of the short arm of chromosome 7D (7DS), we constructed *de novo* genome map of the 7DS arm using BioNano IRYS platform. The mapping was based on direct visualization of sequence motifs (7-bp recognition sites of Nt.BspQI nickase) on single DNA molecules hundreds to thousands of kilobases in length that were prepared from flow-sorted 7DS arm. High frequency of Nt.BspQI recognition sites (~13 nicks per 100 kb wheat DNA) together with high integrity of the chromosomal DNA enabled construction of a high-resolution map covering 92% of the estimated arm length. The map consists of 371 fragments with average length of 0.9 Mb and N50 of 1.3 Mb. The genome map has been anchored to available sequence assemblies of 7DS BAC clones obtained from Illumina pair-end sequencing. Anchoring the 7DS map to a contiguous sequence of ~700 kb indicated several discrepancies between the BAC assemblies and the BioNano map which provided suggestions for assembly improvement. Analysis of 67 assembled BAC clones across the 7DS arm is underway to verify both the clone assemblies and the genome map. Hitherto obtained results suggest that the BioNano maps derived from individual wheat chromosomes/arms will become an affordable tool to support sequence assembly and scaffolding and lead to the production of improved pseudomolecules.

The work has been supported by Czech Science Foundation (P501/12/2554), the Australian Research Council (LP110100200) and Australia Awards.

An integrated approach for the physical mapping of wheat chromosome 5A

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An integrated physical and genetic map represents a prerequisite for DNA sequence assembly of wheat chromosome 5A. 44,744 BAC clones for 5AS, and 51,072 BAC clones for 5AL were fingerprinted using SnaPshot method and the useful fingerprints were assembled into contigs using both FPC and LTC software. Two minimal tiling paths (MTPs), were defined: FPC-MTP consisting of 4,201 for 5AS and 6,560 for 5AL overlapping BAC clones and LTC-MTP made of 5,412 for 5AS and 8,709 for 5AL overlapping BACs. Both MTPs were organized in three dimensional (3D) pools, to increase the efficiency of further anchoring, that was performed using different strategies.

The anchoring of FPC-MTP was done through screening with several classes of molecular markers mapped on four segregating populations, belonging to different species of *Triticum* genus. The categories of the employed molecular markers include ESTs (expressed sequence tags), COSs (conserved ortholog sets), TE (transposable element)-junction based markers and SSRs (simple sequence repeats), these latter obtained either from literature or by *in silico* screening on 454 sequences (2x coverage) of 5AS flow sorted DNA.

To anchor the LTC-MTP an Agilent 15K specific array was produced using probes derived from the 5A Genome Zipper (Vitulo et al, PloS One 2011), all available ESTs mapped on 5A and all markers already genetically mapped on this chromosome. Moreover a set of SNPs (Single Nucleotide Polymorphisms) mapped onto a genetic map derived from a cross between Chinese spring and Chinese spring 5A-*dicoccoides* and developed thanks to the functional Infinium array 90K (Illumina) have been anchored to LTC-MTP as well. This map has been developed as a scaffold to build a dense neighbor map using the other four genetics maps developed for 5A, very useful tool to assist the creation of the physical map. Almost all mapped markers have been assigned to 5A deletion bins, included about 200 ISBPs (Insertion Site Based Polymorphisms) developed from the BAC-end sequences belonging to the FPC-MTP.

The information deriving from these anchoring has been merged, thanks to the partial overlapping between the two MTPs. The update of growing physical map of 5A chromosome 5A is presented.

MAGIC wheat: Multi-parent populations for the genetic dissection of agronomic traits

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The advent of high-throughput genotyping platforms in crop plants means that genetic marker coverage is no longer the major limiting factor in quantitative trait loci (QTL) analyses. Accordingly, experimental focus is now turning to the genetic mapping population design. Multi-parent Advanced Generation Inter-Cross (MAGIC) populations, which combine high allelic input (via multiple parents) and high genetic recombination (via inter-crossing over multiple generations) are emerging as one of the front-runners in the new generation of genetic mapping platforms.

We describe the creation and use of an 'Elite' eight-parent MAGIC population consisting of 1,091 F_7 lines of winter-sown wheat (*Triticum aestivum* L.). After genotyping with a 90,000 single nucleotide polymorphism (SNP) array, analyses find the population to be well suited as a platform for fine-mapping quantitative trait loci (QTL) and gene isolation: patterns of linkage disequilibrium (LD) show the population to be highly recombined, with genetic marker diversity in comparison to a bi-parental cross and an association mapping population found to be 142% and 94%, respectively. We demonstrate the potential of the MAGIC resource by investigating resistance to the major foliar pathogen *Puccinia striiformis* f. sp. *tritici* (wheat yellow or stripe rust), identifying transgressively inherited resistance controlled by three QTL on the long arms of chromosomes 2D, 2A and 1A. The QTL of largest effect mapped to a 3.6 cM interval on 2DL. We go on to identify a highly diagnostic marker for awn presence/absence on chromosome 5BL, which is also associated with fusarium resistance.

The high allelic diversity and genetic recombination captured by MAGIC populations improves the sampling and re-assortment of genetic diversity, aiding the analysis of interacting or complex traits within a unified mapping population. Combined with the suitability for the generation of high density genetic maps, these factors make MAGIC populations ideal platforms for community-based resources for crop improvement, genetic dissection of QTL, and the anchoring of physical-genetic maps.

The Whole Genome Profiling™ for physical mapping of wheat chromosome 6A

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As part of the international wheat genome sequencing consortium (IWGSC; http://www.wheatgenome.org), we developed a sequence-based physical map of wheat chromosome 6A utilizing Whole Genome Profiling (WGP TM) (Keygene, http://keygene.com). A total number of 24,576 BACs (6AS) and 22,656 (6AL) served as inputs for WGPTM. Two different Bacterial Artificial Chromosome (BAC) contig assembly tools, FingerPrinted Contigs (FPC) and Linear Topological Contig (LTC) software were used. The resulted assemblies of the two tools were compared and visually inspected. This visual investigation revealed a highly robust assembly made by LTC compared to that of FPC. Thus, all LTC-built contigs containing at least two BACs per contig were selected. These assemblies contained 1217 contigs for 6AS and 1113 contigs for 6AL, with an L50 of 1 Mb.. In order to facilitate an in-silico anchoring approach, the WGP tags underlying the BAC assemblies were extended by publicly available wheat sequence information. This resulted in an increase of the cumulative sequence information per physical contigs from 2144 nt to 11,067 nt. The extended sequence data were used for the in-silico anchoring against wheat and barley genetic markers with known sequences by which almost 79% of the physical map could be anchored. Moreover, the assigned sequence information led to “decoration” of the respective physical map with 3359 genes. This physical map will serve as the framework for sequencing of chromosome 6A and is of use for map based isolation of important genes/ QTLs located on this wheat chromosome.

A draft sequence of bread wheat chromosome 7B based on individual MTP BAC sequencing using pair end and mate pair libraries

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The Minimum Tiling Path (MTP) for chromosome 7B consists of 3338 and 5561 BACs for the short and long arm, respectively. The total length of the short arm sequence is 425 Mbp (118% of estimated length) and the long arm 524 Mbp (98%). The current assembly for the short arm consist of 103 super-contigs with an average of 33 BACs (max 194, min 2) and 148 BACs (max 219, min 1) for the long arm. Nearly 80% of the super-contigs have been mapped to the gentic map of 7B. The final assembly to be presented is based on re-sequencing of MTP BACs to yield less than 5% of BACs with lower than 20X coverage. In addition, we have sequenced 10 and 20 kb insert mate pair libraries for pools of 12 neighboring BACs in the 7B MTP. We will present a comparison of the 7B assembly with the Triticacae consensus map for 7B. Current analysis include a study of co-regulated 7B genes that are identified by RNAseq for each cell type in the developing endosperm.

Table 1.Summary statistics of MTP BAC assemblies, cont´d

Statistics	Assembly v1 OLC			
	7BS		7BL	
	20% low coverage	5% low coverage	20% low coverage	5% low coverage
Total size	337 Mbp		550 Mbp	580 Mbp
Scaffold #	55,109		72,020	71,000
Max scaffold size	274 Kb		597 Kb	600 Kb
N50 scaffold size	23 Kb		36 Kb	46 Kb
Mean scaffold size	6.1 Kb		7.6 Kb	7.2 Kb
BACs per scaffold	15		13	13

Table 2.Summary statistics of MTP BAC assemblies

Statistics	7BS	7BL
Number of MTP BAC clones	3338	5561
Number of MTP supercontigs	103	148
Clones per MTP-supercontig	33	38
Max. MTP-supercontig size	194	219
Min. MTP-supercontig size	2	1
Total Kbp	425261	529407
Arm coverage*	118%	98%

Session I

Genetic Resources

Seeds of Discovery (SeeD): A project to systematically characterize and mobilize novel genetic variation from genebanks into breeding programs

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Advancements in DNA-sequencing technologies are providing an opportunity to unlock the genetic potential of genebank collections by assisting breeders and researchers to accelerate the development of high-yielding, climate-ready cultivars. The *Seeds of Discovery* (SeeD) project strives to capture this opportunity to systematically mobilize novel, beneficial genetic variation into maize and wheat breeding programs (http://seedsofdiscovery.org). We are on our way towards genome-profiling approximately 40,000 maize and 160,000 what accessions from genebanks of global importance. Genotyping-by-sequencing (GbS) is our method of choice because it minimizes ascertainment bias, a key feature when characterizing underexplored genepools. We use two complementary GbS 'flavors' for different applications: the systematic diversity surveys of maize and wheat genebanks are performed using the DArTseq approach (higher sequencing depth, lower multiplexing level), while the Cornell-University approach (lower sequencing depth, higher multiplexing level) is used for maize association mapping. We link molecular data to traits typically targeted by breeders by evaluating selected accessions under field conditions for characters like heat and drought tolerance, P and N efficiency, grain-quality features, and resistance to selected diseases and pests. Genetically diverse 'allele-donor accessions' with favorable trait values are selected to develop 'bridging germplasm' harboring novel alleles in breeder-ready genetic backgrounds. In the case of wheat, we are developing a linked-topcross population (LTP) panel of 10,000 lines by introgressing exotic genome segments from 200 genebank accessions into a common set of ten elite lines, using a single-round robin, partial-diallel design to link individual topcross families via common elite lines. In maize, we pursue different introgression approaches for oligo and polygenic traits, which range from generating doubled haploids from landraces to address short-term needs, to genomic-selection approaches informed by *in silico* simulations to develop more breeder-ready germplasm enriched for alleles conferring desirable variation for polygenic traits. We will present an overview of the project, including ongoing genotyping, phenotyping, pre-breeding and software-development efforts.

Unravelling of genetic diversity and domestication trends for allele discovery in a bread wheat landrace collection

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The A.E. Watkins bread wheat collection (826 accessions) was collected in the 1930s from a near global distribution. It is now developed into resources for research and breeding as part of the WGIN (www.wgin.org.uk) and WISP (www.wheatisp.org) project. Genotyping of the collection was conducted in order to assess the levels of genetic diversity present. Furthermore, an analysis of the population structure has revealed that accessions came from nine ancestral populations, which can be roughly matched to geographic regions (Wingen et. al. 2014). To make the genetic diversity of the collection more accessible, 26 bi-parental mapping populations from diverse landrace accessions were developed, SNP genotyped and mapped. Each population shares the same elite parent, 'Paragon'. These mapping populations are available for QTL mapping and detection of novel alleles. Additionally, these populations give access to interesting questions regarding the evolution of wheat before and after domestication. The maps were integrated into a single consensus map together with maps from elite mapping populations, the most popular of which is the Avalon x Cadenza UK reference population. Maps are compared to the consensus map in order to disclose domestication trends. Moreover, the identification of markers that show segregation distortion may give insights into selection patterns. An analysis of frequency of QTL co-localisations will give insight into the genetic architecture of traits in wheat. A better knowledge of evolutionary trends and selection processes, which have shaped wheat in the past, may guide the design of breeding strategies in the future. The Watkins collection and the set of bi-parental populations will be developed further into a nested association mapping (NAM) panel containing 85 mapping populations.

The project involves collaborations with M. Hawkesford (Rothamsted Research), K. Edwards (Bristol University) and J. Folkes (Nottingham University).

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Seed conservation in *ex situ* genebanks – genetic and biochemical markers of viability illuminate mechanisms of seed longevity in barley

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The Global Strategy for Plant Conservation, a key matter of the Convention on Biological Diversity (CBD), illustrates that our plant genetic resources have been given a priority status and need to be conserved by *in situ* and *ex situ* approaches. Due to lower cost and better control especially seed conservation is mainly used for most of the 7.4 Mio worldwide stored genebank accessions. However, the ability of seeds to survive a certain period of time, termed seed longevity, is strongly dependent on the growth conditions of the mother plant, pre-storage and storage conditions and the genetic background.

The present study will provide deep insights into seed longevity of barley (*Hordeum vulgare* L.) which is among the four most important cereals and conserved with approximately 21,000 accessions at the Federal *Ex situ* Genebank at Gatersleben. Its projected half-life is estimated at 7 to 9 years at ambient conditions (20°C, 50% RH) and at sub-zero temperatures (-18°C, 11% RH) this can rise to 84 years. However, barley seed longevity differs intraspecifically. After 34 years of storage at 0 °C, most accessions maintained high viability (> 90 %), but in some accessions viability declined to below 50%. Modern quantitative genetic tools unlock the background of different shelf lives. On basis of five mapping populations using association and QTL mapping und different storage treatment, ranging from dry, long-term stored material to experimental seed ageing using high oxygen pressure, major QTLs were discovered. On chromosomes 2H, 5H and 7H, QTL and marker sequences could be related to known functions described for rice, wheat, soybean or Arabidopsis orthologues influencing mechanism of plant development, biotic and abiotic stress response.

To address aspects of abiotic, including oxidative, stress, two major antioxidant groups were analysed. No correlation was found between seed ageing and the lipid-soluble tocochromanols. Conversely, changes in the water-soluble glutathione, and related thiols, indicated a strong shift towards more oxidising intracellular conditions in seeds subjected to long-term dry storage at two temperatures and to two experimentally ageing treatments.

Quantitative variation of barley culm length and sturdiness is controlled by *BREVIARISTATUM*, *BRACHYTIC* and *ERECTOIDES* gene loci involved in brassinosteroid hormone metabolism

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Culm length and sturdiness are quantitative characters important for securing cereal crop yield in adverse weather conditions. Brassinosteroids, a class of growth-promoting hormones, are known to regulate the architecture and height of plants. We recently connected a unique combination of phenotypic traits, including short and sturdy culm, with deficiencies in brassinosteroid metabolism in semi-dwarf mutants of barley (*Hordeum vulgare* L.). A comprehensive phenotypic screen of 160 near-isogenic short-culm mutant lines from the *brachytic*, *breviaristatum*, *dense spike*, *erectoides*, *semi-dwarf*, and *slender dwarf* mutant groups, identified candidate mutants with potential defects in brassinosteroid biosynthesis and signaling. *In silico* mapping of brassinosteroid-related genes in the barley genome in combination with re-sequencing of candidate mutant lines assigned more than 20 historic mutants to three brassinosteroid-biosynthesis genes (*HvBRD*, *HvCPD*, *HvDIM*) and to the brassinosteroid receptor gene (*HvBRI1*). Subsequent analyses of F_2- and M_2-populations, allelic crosses and modeling of non-synonymous amino-acid exchanges in protein crystal structures give further understanding of the control of barley plant architecture and sturdiness by brassinosteroid-related genes. The identified mutants represent alternatives to the widely used but highly temperature-sensitive *uzu1.a* allele of *HvBRI1* for the development of sturdy and climate-tolerant barley cultivars.

Allele mining in wild barley: Finding new exotic genes which control flowering time in the barley nested association mapping (NAM) population HEB-25

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Flowering time of crop plants is crucial in agriculture, especially to cope with the current challenges in agriculture where biotic and abiotic stresses threaten future productivity. Plant breeders have to deal with this problem and take it into account in upcoming breeding programs. Extending biodiversity is a key instrument to enhance adaptation of cultivated barley to environmental changes. In this regard, we recently developed the barley nested association mapping (NAM) population HEB-25 (Halle Exotic Barley). HEB-25 consists of 1,420 BC1S3 lines, sub-divided into 25 families, which originate from crosses of the elite spring barley cultivar Barke with 25 wild barley donors (*Hordeum vulgare* ssp. *spontaneum* and *H. agriocrithon*). HEB-25 is well suited to identify, map and, ultimately, clone genes controlling agronomic traits and to transfer favorable exotic alleles into the elite barley gene pool.

The aim of the present project was to find new genes and allelic variants that control flowering time. The identification of quantitative trait loci (QTL) associated with flowering time was performed through a genome wide association study (GWAS). To this end, genotype data were derived from the Infinium 9k iSelect HD chip for barley, which consists of 7,864 SNPs (5,709 are polymorphic in HEB-25). In parallel, phenotype data on flowering time were collected for HEB-25 in three field trials from 2011 to 2013 at the Julius-Kühn experimental field station in Halle, Germany. Subsequently, both data sets were merged to conduct a GWAS.

The GWAS revealed QTL on all seven chromosomes. Both, known regulators of flowering time (e.g. *Ppd-H1*) and new loci could be identified. The effect of single exotic alleles on days to flowering ranged from a reduction of 9 days to a delay of 9 days. These results show that the statistical power to detect QTL in HEB-25 is high and reveal the high potential of exotic genes affecting flowering time.

Genetic basis of photoperiod-insensitivity in barley

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Onset of flowering depends on when and how the environmental cues are perceived during the seasonal changes. Barley is sensitive to photoperiod, but mutation in photoperiod-related genes led to photoperiod insensitivity (reduced photoperiod responsiveness). Thus the distribution range of cultivated barley extended to diverse geographical regions including higher latitudes. In order to investigate the genes involved in adaptation to these diverse environments, 224 barley accessions of world-wide origin were phenotyped for heading date in four diverse geographical locations (viz., Germany, Turkey, Syria and USA). Genome-wide association scans were performed using 9K iSelect SNP markers. Highly significant associations were for instance observed at the *Ppd-H1* genetic region for Germany. Since, the *ppd-H1* mutation was an important event in barley evolution under domestication i.e. involved in extending the distribution range of domesticated barley beyond the wild progenitor range, we re-sequenced the *Ppd-H1* locus in these 224 accessions and discovered the causative SNP. Furthermore, to uncover the origin of photoperiod insensitivity in barley -- which was often debated - we re-sequenced the *Ppd-H1* locus in 2057 geo-referenced wild and cultivated barleys. Contrary to the previous findings, we discovered that photoperiod insensitivity originated after the initial domestication, outside the Fertile Crescent.

Diversity in heritage barleys: Adaptation on a local scale

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Most of the work examining adaptation in barley has focused on the wild progenitor *Hordeum vulgare* subsp. *spontaneum* sampled across its wide natural distribution. Although these studies have enhanced our understanding of the dynamics of genetic diversity, the impact on contemporary breeding has still to be realised. To improve and stabilise yield under less intensive production systems and changing environmental conditions requires an understanding of adaptation at the local scale. We have sourced a collection of locally adapted 'heritage' accessions from the UK and Scandinavia, most of which have been grown for hundreds of years, surviving both changes in climate and agricultural practise. These accessions have been genotyped with 1536 SNPs and phenotyped for life history traits under field conditions over several seasons. Genome wide analysis revealed very different patterns of diversity between the heritage accessions and current cultivated lines. Many of the more Northern UK accessions collectively known as 'bere' barley grouped with Scandinavian accessions suggesting a possible Scandic origin contrary to previous results. An initial association analysis using heading date and thousand grain weight revealed several QTLs some of which were previously identified from other studies and other novel QTLs worthy of further investigation.

The barley meiotic tool box: Fundamental science for practical breeding

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Breeding work relies fundamentally on recombination but the control of this process is not fully understood especially in crop plants. In barley (and wheat) the distribution of meiotic crossover events is highly skewed meaning that, substantial proportions of the chromosomes are inherited together as large linkage blocks, preventing the generation of novel gene combinations and useful variation that could be exploited in breeding programmes. An ability to modify the pattern of recombination in these species could therefore have profound impact on the breeding of the crops. Meiosis is highly conserved across eukaryotes and has been temporally and physically dissected in detail in yeast and mammalian systems through the use of meiotic mutants and novel technology. Studies based on the model plant Arabidopsis have been developed reveal that although the mechanism of meiosis is conserved, the control of crossing over is potentially different to that found in mammals. Recent work has shown that meiosis in barley differs from *Arabidopsis* and it is becoming evident that specific strategies are needed to be developed in this large genome cereal. One current strategy is to fully characterize our collection of 14 non-allelic desynaptic (*des)* mutants that exhibit perturbed meiosis and semi-sterility compared to wild type. A number of these mutants are currently being characterized by genetic mapping using their semi-sterility phenotype but for a detailed understanding it is essential that cytological analysis (FISH and Immunocytology) of the meiosis in the mutants is also carried out to assess the effect of the mutation on chromosome pairing and recombination. Using advances in bio-imaging (OMX 3D-SIM), some interesting and potentially informative differences in the behaviour of these desynaptic mutants have been revealed and will be discussed.

Germplasm development in the landrace "Pillar" of the Wheat Improvement Strategic Programme

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The Wheat Improvement Strategic Programme (WISP) consortium is a BBSRC funded collaborative programme bringing together experts in wheat genetics and breeding from five institutions. WISP is a comprehensive pre-breeding programme - the first of its kind in over 20 years - aiming to guarantee the sustainability of wheat production against the background of growing global population and changing environment.

Specific goals of the project are to understand the genetics behind factors limiting grain yield, such as drought tolerance, plant shape and resistance to pests and diseases. To identify new and useful genetic variation from related species and sources of wheat germplasm not adapted to target environments. Cross wheat lines to produce germplasm that allows the identification of genes influencing key traits and generate a database of genetic markers for use in precision breeding.

The good news for growers is that both the new germplasm and the information generated by this project will be made freely available. That means plant breeders can use the germplasm to cross with their existing lines, while academics will be able to make use of it to understand the mechanistic basis of key traits in bread wheat.

Using the A.E. Watkins landrace collection at the John Innes Centre, which was collated in the 1930s from some 32 countries around the world, we believe important genes ‘lost’ from modern breeding have been discovered. Along with these landraces and the UK bread Wheat variety Paragon we have developed 85 mapping populations for study. This large scale germplasm development, subsequent phenotyping and large scale genotyping has allowed the dissection of novel genetic variation for traits that are available in the landraces but not yet deployed in current UK germplasm.

The project involves a consortium made up of the John Innes Centre Norwich, Rothamsted Research Harpenden, NIAB Cambridge, Bristol University and Nottingham University. The main project website is http://www.wheatisp.org/

Evaluation of flowering time, β-glucan content and tillering of wheat/barley introgression lines

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Crosses between wheat and barley, two of the most important cultivated cereals, could make it possible to incorporate the earliness, favourable amino acid composition, dietary fiber content, salt and drought tolerance and good tillering ability of barley into wheat. New wheat/winter barley addition lines developed in two combinations (Mv9 kr1/Igri, Asakaze/Manas) were used to study the effect of individual barley chromosomes on agronomic traits in wheat background and to induce wheat-barley translocation lines. The flowering time of the addition lines was analysed in phytotron and the field. The 7H addition line was the earliest and the 4H addition line the latest in both cultivar combination. The β-glucan content and the salt tolerance of the Asakaze/Manas 7H addition line was higher than that of the wheat parent. The morhological traits of the addition lines were described in details in the phytotron and in the field. Wheat-barley translocation lines were induced using several methods: irradiation, multiplication in tissue culture, 2C gametocid system and by crossing with the CS *ph* mutant. The 5HS-7DS.7DL translocation was used for physical mapping of 7D and 5H chromosomes. The 4BS.7HL translocation line had higher β- glucan content than the wheat parent and it was used for mapping the *HvCslF6* gene in the centromeric region of 7HL. The 3HS.3BL translocation was transferred into a modern Martonvasar wheat cultivar (Mv Bodri) thus the plant height was significantly reduced. The tillering of the 3HS.3BL line was better and the other yield components were similar to the controll wheat variety in two consecutive years in the field. The morhological traits of several other homozygous, fertile translocation lines were analysed (2DS.2DL-1HS, 6BS.6BL-4H, 5HS-4DS.4DL, 4HL.5DL, 2HL.3BL). Ditelosomic addition lines containing 10 barley chromosome arms from Manas barley cultivar were developed. The effect of individual chromosome arms on morphological and agronomic traits is studied in the phytotron and in the field. The ditelosomic addition lines are good starting materials to produce wheat/barley centric fusions and for flow-sorting of individual barley chromosome arms from Manas barley.

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Session II

Grain Yield

Looking for new engines of yield improvement

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A substantial genetic gain in wheat grain yield was observed worldwide in the second half of the 20th century. This increase was due mainly to the higher grain number per unit area. Changes in thousand-kernel weight – another major component of the grain yield – were not consistent in many regions of the world. The introgression of major dwarfing genes for plant height represented a significant step towards modifying the plant architecture of modern high-yielding cultivars with better lodging resistance and higher yield potential. As a consequence, the harvest index – the ratio of grain weight to aboveground biomass – was increased. The total aboveground biomass of old and modern wheats remained constant, and similar results were obtained when comparing the root systems at anthesis.

After this successful period of yield improvement, wheat yields have been stagnating or decreasing in Europe over the last two decades. Among the traits with a direct influence on yield performance, the harvest index and plant height have reached the optimum range in most regions. It has become evident that wheat breeders will have to face new challenges if they are to find new ideotypes suitable for new management systems and to overcome adverse climatic conditions to narrow the increasing yield gap, while also improving the yield. One option to increase yield potential would be to maintain the harvest index at a higher level of biomass production. In the light of climate change and the new pesticide regulations introduced by the EU, resistance breeding is an important indirect tool for increasing yields. Molecular tools contribute to the better utilization of the wild and cultivated relatives of wheat as potential genetic resources. Wheat breeders are again focusing on heterosis breeding and hybrid wheat selection. Using new technologies it will be realistic to study genes controlling complex traits in order to identify the best alleles for future yield improvement.

Hybrid breeding in wheat

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Hybrid breeding is a remarkable success story in several allogamous species. The main advantages of hybrid versus line varieties are increased trait values due to the exploitation of heterosis, larger yield stability especially in marginal environments, and the ease of stacking dominant major genes. We examined 1.604 single-cross wheat hybrids and their 135 parental lines in field trials for grain yield, plant height, flowering time, biotic stress resistances, frost tolerance, as well as quality traits. We observed that hybrids were for most traits superior to the mean of their parents. Furthermore, we found that hybrids outperformed their parents with respect to their yield stability. This clearly underlines the potential to improve stress resistance switching from line to hybrid breeding. One important challenge in hybrid wheat breeding is the development of accurate methods to predict hybrid performance before evaluating crosses in intensive field trials. We developed association mapping, ridge regression best linear unbiased prediction (RR-BLUP), Bayes-A, Bayes-B, Bayes-C, and Bayes-Cπ approaches to predict hybrid wheat performance. Moreover, we bridged the gap between marker-assisted and genomic selection implementing weighted RR-BLUP. The accuracy of the developed prediction approaches were studied using the phenotypic data in combination with 9k and 90k SNP array data. The high cross validated accuracies clearly underlines the potential of genomics based prediction of hybrid performance in wheat.

Breeding progress and trends of winter wheat yield in Germany

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Bread wheat has an outstanding global importance for agriculture and human nutrition. Therefore, growers are interested in high and stable wheat yields. Likewise, processors and end users are interested in sufficient and sustainable production of seeds, flour and final products. Therefore, it is alarming that farm yields of wheat in major growing regions were reported to stagnate recently. For that reason, a couple of years ago we have started repeated field experiments with a sample of 90 winter wheat varieties registered in Germany during the last decades, i.e. from 1966 to 2007. The initial study was run for 3 years at 5 locations with two nitrogen levels. The cultivars' performance regarding grain yield, yield components and response to various fungal diseases was analyzed.

The experimental results show that the genotype-specific performance of wheat has been steadily increasing. The yield progress is mainly due to a significant increase of the major component GRAIN NUMBER PER SPIKE. In contrast, the components number of SPIKES PER UNIT AREA and the 1000-GRAIN WEIGHT remained relatively stable over the observed time period. The combined genetic improvement of grain yield has been calculated at about 37 kg per ha and year, accompanied by a generally better resistance to diseases such as powdery mildew, leaf rust and Septoria leaf blotch. Recent cultivars also tend to be more resistant against Fusarium head blight. In addition, new varieties are generally earlier, shorter and more lodging resistant, determining a better crop stability. Whereas many of these (simple) traits have been genetically analyzed in-depth, the complex yield trait still remains to be elucidated genetically.

On the basis of the results of exact field trials, several reasons are discussed for the observed stagnation of winter wheat farm yields in Germany. Possible causes include the expansion of winter wheat growing areas (narrow rotations), elevated temperatures during the grain filling period, etc. However, our results demonstrate that the stagnation of wheat yield in practice is not due to stagnancy in the genetic improvement of the crop, even though the initial study included line varieties only. Since hybrids have been released recently and tend to be superior to lines, extended winter wheat field trials have been started, including hybrid cultivars. These experiments are expected to deliver new insights into the prospects and possible limits of enhancing wheat performance in the future.

Genetic analysis of pre-flowering development in barley (*Hordeum vulgare* L.)

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Optimal flowering time is one of the important strategies used in breeding programs to acclimatize crops to environment and subsequently achieving high yield potential. The pre-flowering phase is one of the most important phases and has direct impact on yield potential and final grain yield. Maximum number of spikelet primordia per spike (maximum yield potential) and its abortion are the major events during this phase (Alqudah and Schnurbusch, *Funct Plant Biol* 2014, doi.org/10.1071/FP13248). The pre-flowering phase can be divided into three sub-phases: 1) Leaf initiation; 2) Spikelet initiation; and 3) Spike growth phases. The lengths of these phases are affected by environmental conditions such as photoperiod and vernalization as well as genotypic constitution. Such factors directly contribute to reach the final time to flowering and yield potential. Hence, genetic analysis of pre-flowering phases in cereals is necessary at this time point to better understand the role of these sub-phases in increasing yield. We used a genome-wide association study of a world-wide spring barley collection to detect the genetic basis of pre-flowering development and its role in yield potential and final grain yield. Through genetic associations we identified new QTLs for growth and developmental traits, including several known flowering time genes. This study provides great power for understanding the genetic variation of flowering time. Here, we propose a new genetic network model for barley flowering time, which includes several newly, identified genes for barley's flowering time pathways such as the *HvCONSTANS*-like genes.

VRS3: A fourth piece in the barley row type puzzle

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The evolution of row type has long been a source of debate in barley, with six-rowed types even suggested as a novel species in the early 20th century. Currently in the UK, six-rowed barley is restricted to the feed market due to its non-uniform grain size resulting in higher levels of screenings and uneven starch modification during the malting process. However, in some other countries, the criteria for selecting malting barley are different, with six-rowed cultivars widely used, e.g. France and USA.

Our overall aim is to use improved understanding of the network of genes involved in row-type determination to develop six-rowed barley with enhanced yield and more even grain characteristics. Row type is determined by the fertility of the two lateral florets in the developing spike. Mutation studies have identified twelve regions of the barley genome which result in varying levels of lateral floret fertility. Three of the twelve loci *VRS1*, *VRS4* and *INT-C* have been intensively studied and the underlying genes identified as *Hv*HDZIPI (2H), *Hv*Ra2(3H) and *Hv*TB1(4H) respectively. Currently, all high yielding six-rowed cultivars have the *vrs1*.a allele in combination with the *Int-c*.a allele. This combination yields treble the grain number per tiller, combined with a significant reduction in the number of tillers per plant compared to two-rowed cultivars.

Here we present our work using F2 mapping populations and mutant barley collections to successfully locate, identify and characterise *VRS3*, a fourth locus involved in the switch between two-rowed and six-rowed barley.

Cloning and characterization of a semi dwarfing gene in barley

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Our goal was to identify a barley semi dwarfing gene of which several recessive alleles are known. It had been showed previously that this semi dwarfing locus reduces lodging without causing significant yield reduction.

To identify candidate genes we used marker sequences flanking the semi dwarfing locus in barley in an *in silico* approach. Thereby we identified the corresponding genomic regions in *Oryza sativa, Sorghum bicolor* and *Brachypodium distachyon*. The gene order in the region is highly conserved between organisms; however we could not identify a candidate gene carrying mutations in all allelic lines.

To explore if the respective genomic region in barley contains additional, non syntenic genes we used the genes identified in the *in silico* approach in a PCR based BAC pool screening of the HVVMRXALLeA barley BAC library. Identified BAC clones could be cross referenced to overlapping sequenced BAC clones of the HVVMRX83KHA BAC library, which is part of the barley genome map. Indeed the region of interest in barley contains a gene involved in signal transduction that is absent from the syntenic regions in *O. sativa, S. bicolor*, and *B. distachyon*. Severe mutations in this gene could be identified in all lines carrying an allele of the semi dwarfing gene.

Some of these alleles are further available as near isogenic lines (NILs) in the background of the two row spring cultivar Bowman. All NILs carry the same mutation as in the respective original mutant. Due to the recurrent backcrossing following the semi dwarf phenotype this can be regarded as genetic evidence that the identified gene is the locus of interest in barley.

This study is an example for how combining the knowledge provided by the research community like the mutant collections in gene banks, the near isogenic lines, the BAC libraries and the genome sequencing effort can efficiently be combined to clone mutant genes in barley.

A multiparental cross population for mapping of QTL for agronomic traits in durum wheat (*Triticum durum* Desf.)

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Multiparental cross designs for mapping quantitative trait loci (QTL) in crops are efficient alternatives to conventional biparental crosses because they exploit a broader genetic basis and are characterized by higher mapping resolution. By crossing four elite cultivars, we have developed a multi-way recombinant inbred line (RIL) population in durum wheat (*Triticum durum* Desf.). A linkage map spanning 2,663 cM and including 7,594 single nucleotide polymorphisms (SNPs) was produced by genotyping 338 RILs with a wheat-dedicated 90K SNP array based on phenotypic data collected over four field experiments. QTL analysis was carried out for a number of agronomic traits including grain yield, by both interval mapping on reconstructed haplotypes and bi-allelic test. For all traits, functionally different QTL alleles, in terms of direction and size of genetic effect, were distributed among the four founders. However, the pattern of identity by descent between the founders was shown to influence the informativeness of the analysis. For the combined data across environments, two yield QTL were mapped on chromosomes 7A and 2B, the former co-mapping with the vernalization locus *Vrn*-3 while the latter mapped independently from phenological traits hence representing a yield *per se* QTL. This multiparental cross population provides the wheat community with a highly informative resource for the dissection of the genetic architecture of multiple agronomic and adaptively relevant traits.

Towards positional cloning of a grain yield QTL on chromosome 3B in wheat (*Triticum aestivum* L.)

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Drought and high temperatures occur independently or simultaneously during the wheat (*Triticum aestivum L.*) life cycle and can severely reduce yield. Grain yield is determined by complex interactions between the genetic of the plant and the nature and timing of abiotic stress. Identifying new genes is essential to increase drought and/or heat tolerance for future grain yield improvement. Quantitative trait loci (QTL) analysis, fine mapping and wheat genome sequencing information are essential tools when using forward genetic approaches to tackle this challenge. Grain yield was studied in two doubled haploid populations (RAC875/Kukri and Excalibur/Kukri) and two recombinant inbred line populations (RAC875/Kukri and Gladius/Drysdale) to fine map a QTL named *qYDH.3BL* and located on the long arm of chromosome 3B. The alleles carried by RAC875, Excalibur or Drysdale improved grain yield by between 5% and 12.5%. A fine genetic map was generated for *qYDH.3BL* using the chromosome 3B sequencing database (3Bseq project http://urgi.versailles.inra.fr/) and the Bioplatforms Australia Wheat Sequencing Initiative data (http://www.bioplatforms.com.au/). The gene space was defined using the chromosome 3B reference sequence and gene expression was analysed using the TC Harvard database http://compbio.dfci.harvard.edu/ and the Triticeae sequence database TriFLDB (http://trifldb.psc.riken.jp/). We identified a preliminary list of candidate genes and large deletion/insertions at the *qYDH.3BL* locus. These results open new perspectives to potentially improve wheat grain yield under high temperatures and water deficit.

BWGS: A Breed Wheat Genomic Selection pipeline

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Wheat is the most widely grown crop in the world, representing a major renewable resource for food, feed, and industrial raw materials (Charmet 2011). Genomic selection (GS) is a very powerful tool for improving wheat traits such as grain yield, protein and gluten content and thousand kernel weight. GS is focused on the methods for estimating the Genomic Estimates of Breeding Values (GEBVs) using dense molecular markers spanning the entire genome (Meuwissen et al. 2001) that promises to accelerate rates of genetic gain (Rutkoski et al. 2013). The BreedWheat project (France, 2011-2019) (http://www.breedwheat.fr) aims to develop a new genome-to-phenotype multi-model machine learning pipeline for GS, called BWGS. The pipeline [Fig. 1] consists of four models for marker reduction (RMR - Random Marker Reconstruction, LD - Linkage Disequilibrium, GMSLD - Genotypic Matrix Split with Linkage Disequilibrium, RM - Relatedness Matrix), six models of genotype imputation (MNI - MeaN Imputation, kNNI - K Nearest Neighbour, SVDI - Singular Value Decomposition, EM - Expectation Maximization, Random Forest, LWI - Locally Weighted Linear Regression) and seven models of breeding value prediction (RR - Ridge Regression, SVM - Support Vector Machine, RKHS - Reproducing kernel Hilbert space, LASSO - Least Absolute Shrinkage and selection Operator, EN - Elastic-Net, RF - Random Forest, GBLUP - Random Regression Best Linear Unbiased Predictor) were implemented.

The pipeline can be applied for cross-validation (CV), model comparison and prediction or used separately these functions for many different studies in GS.

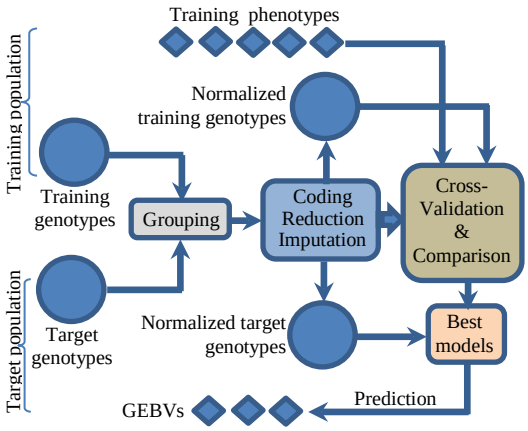

Figure 1: Schema of BWGS pipeline

The CV test using RMR, LD and RM reduction with MNI imputation and seven prediction models for INRA dataset shows the correlations between 0.35±0.02 and 0.51±0.02. It shows that using 2236 DArT markers return better accuracy than the 106 045 SNPs. The obtained results by RKHS and RF models are more accurate than by SVM, RR, EN, GBLUP and LASSO. Another test for the Cornell wheat data with all imputation and prediction models shows correlations ranging from 0.27±0.1 to 0.40±0.01. More expressions and results on model comparison and prediction using INRA, CORNELL and CIMMYT wheat data will be added in the full paper of this abstract.

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Analyses of the response of 100 different barley genotypes to future CO_2 concentrations in order to optimize the CO_2 fertilization effect

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The continuing increase in atmospheric CO_2 concentration will have direct implications for plant growth and agricultural ecosystems, as CO_2 is the important resource for plant growth. Plant growth and yield of C3 crops are known to be positively affected by elevated CO_2. Although recent evidence from studies with a small number of genotypes suggest that intraspecific variability exists among genotypes, a systematic evaluation of a broader set of diverse genotypes under field conditions is still lacking. However, sufficient genetic variation is a prerequisite that would allow breeders to select for CO_2 responsiveness. Results of a 2-years field experiment are presented during which a diversity set consisting of 101 barley genotypes was exposed in open-top field chambers to ambient CO_2 (~400 ppm) and elevated CO_2 (~700 ppm) during the growing seasons.

Elevated CO_2 increased yield and above ground biomass by ~23 % and 22 %, respectively, averaged over years and genotypes. The most influenced yield and biomass parameters, respectively, were the kernel number per ear (+13 %) and the stem dry weight (+29 %).

However, there were significant differences between genotypes, indicating a high genetic variability in CO_2 responsiveness in barley.

Furthermore, significant differences in the reaction of 2-rowed and 6-rowed genotypes were detected. While 6-rowed genotypes showed a significant yield increase under elevated CO_2, the increase for 2-rowed genotypes was smaller and statistically not significant.

The variability among the cultivars within the diversity set points to the fact that no indirect selection of CO_2 responsiveness has been conducted so far in barley.

Whole genome association analyses were conducted using a QK mixed model approach. Based on 3842 polymorphic mapped SNPs from the Illumina 9k-chip and phenotypic data obtained in two seasons a total of 134 highly associated (-log p values $\geq$ 3) markers were detected of which 75 were associated with multiple traits.

Results of the association studies and *in silico* mapping of published CO_2 responsive genomic regions were used for the selection of candidate genes which are currently sequenced on a subset of genotypes and will be analyzed for differences in expression.

Session III

Abiotic Stress Resistance

Leveraging genomics approaches to enhance abiotic stress resistance in cereals

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Improving cereal yield in conditions of abiotic stress is a daunting undertaking due to the quantitative nature and complexity of the adaptive response of the plant to unfavourable growing conditions. Although conventional breeding has successfully enhanced resistance to abiotic stress through a direct selection for yield, genomics-assisted breeding (GAB) provides novel opportunities to accelerate such gains through marker-assisted selection targeting beneficial alleles at major QTLs (e.g. via marker-assisted selection) and/or genome-wide selection for unmapped QTLs. Additional opportunities (e.g. genetic engineering) are offered by the cloning of major QTLs controlling the plasticity of morpho-physiological traits (e.g. root architecture) with a key role in cereal adaptation to unfavourable environmental conditions. The first step for deploying locus-specific variability is the identification and characterization of QTLs. Recently, multiparental and association mapping coupled with high-density SNP profiling have streamlined gene/QTL discovery. Among abiotic stresses, drought is the most prevalent and challenging one in view of its economic impact, low heritability and critical role played by phenotyping (Tuberosa 2012, Frontiers Plant Physiology). A number of examples will be presented and critically assessed.

Notwithstanding the plethora of QTLs described in the literature, so far only a handful of major QTLs have contributed toward the release of cereal cultivars with enhanced abiotic stress resilience. A critical factor for a more widespread exploitation of locus-specific GAB is the limited availability of major QTLs with consistent effects across different elite genetic backgrounds, environments and management practices (GxExM; Collins et al. 2008, Plant Phisiology). While QTL cloning will increasingly shed light on the molecular and functional basis of abiotic stress resistance, phenotyping remains a major limiting factor for more effectively leveraging GAB. The growing interest in yield modeling based on QTL effects for morpho-physiological features provides further opportunities for improving cereal performance under unfavourable conditions (Tardieu and Tuberosa 2010, COPB). From an applicative standpoint, a succesful integration between GAB and conventional breeding requires a multidisciplinary approach and stronger public-private partnerships.

Identification of genomic regions involved in frost tolerance in winter rye

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Frost stress compromises crop growth and yield and restricts the geographical distribution of crop production. Rye is the most freezing tolerant small grain cereal and constitutes an excellent model to study the genetic architecture of frost tolerance in cereals. In this study we focus on the identification of genomic regions involved in frost tolerance in winter rye by linkage mapping in a population of recombinant inbred lines (RILs) segregating for frost tolerance. The mapping population was developed from a cross of the elite line Lo157 with one gamete of a highly frost tolerant selected fraction of the Canadian Puma population. The Canadian Puma population exceeds the frost tolerance level of European winter rye and serves as a source of valuable new alleles for introgression of frost tolerance into European elite lines. The mapping population was profiled with 384 SNPs from the Rye-5K array. In each of three years, line *per se* and testcross performance was assessed in field trials in two Russian and two Canadian locations and in controlled freezer tests. Phenotypes were obtained on the traits survival after winter and development after winter in the field trials and on the trait regeneration after frost treatment in the freezer test. QTL analysis based on 1st and 2nd year phenotypic data from lines *per se* and testcrosses identified new frost tolerance QTL and confirmed known QTL. The largest proportion of phenotypic variance was explained by a major QTL on chromosome 5R at the *Fr-2* locus. This region on 5R harbors a cluster of transcription factors of the *Cbf* gene family, whose members are involved in the frost responsive network. Including candidate genes of the frost responsive network into the genetic linkage map will reveal further insights into the genetic basis of frost tolerance in rye. Investigating genetic correlations between line and testcross performance for the traits under study will provide an indication for the suitability of frost tolerance testing on the line *per se* level in breeding programs.

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Growth, wilting and recovery – digital imaging and modelling of vegetative biomass growth in seasonal drought conditions

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An automated high-throughput phenotyping (HTP) imaging-based system was used to phenotype vegetative drought tolerance of barley. The traits obtained from HTP were associated with HT-genotyping data from the 9K barley iSelect array (Comadran & Kilian et al. 2012). Genome-wide association studies (GWAS) were performed to map loci for drought tolerance. In total, 100 two-rowed spring barley genotypes (Pasam et al. 2012) were evaluated with daily imaging (visible light sensor). Well-watered (WW) and drought stress conditions were investigated in three consecutive experiments in 2012 and 2013. In WW-treatment, plants were daily watered to a target weight, while the stress treatment included a drought period of ~ 3 weeks followed by a recovery period of two weeks. Plant growth was estimated using the image analysis pipeline (IAP, Klukas et al. 2013). We estimated plant biomass development by calculating 'digital biomass' - a pixel volume showing a high correlation to plant fresh weight. Stress influence on digital biomass was visualized and quantified. The barley collection offered a broad range of phenotypic variation to stress response. Growth models were applied to biomass curves and revealed several novel drought-related traits. In WW-treatment growth followed a logistic curve – and growth rates, time of maximal growth and maximum vegetative biomass were calculated from the model. Digital biomass, plant height, plant width and compactness were used to qualify the effect of stress on morphological characters and plant growth. A colour classification with different ratios allowed investigating the wilting process. In total, 4,873 SNPs were informative for the barley genotypes. GWAS were computed using a mixed-linear model. Altogether, 48 SNPs were significantly associated (-Log(p)>3) with biomass development over time under WW-conditions. GWAS for early and late biomass development resulted in different loci. Results for drought tolerance-related traits will be presented and discussed.

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Genetic control of plant growth response of wheat to drought

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Drought is a major cause of decrease in worldwide crop production. By coupling physiological and genetic methods we will increase our understanding of the basis of drought tolerance which is required for increasing the genetic yield potential in future varieties. Many quantitative trait loci (QTL) identified for yield and yield components in low rain-fed environment in wheat are actually expressed in specific environmental conditions. By measuring accurately the environmental variables and using ecophysiological models, we can dissect the response to the environment into elementary and simpler traits and identify the conditions where a QTL is specifically expressed. Defining the conditions of expression of a QTL and the physiological mechanism underlying a QTL will enable to understand the function of the underlying gene and use a physiological trait as a proxy for tracking a yield QTL in large genetic populations to make progress toward the QTL cloning. Ecophysiological models also enable to calculate dynamic traits, such as the relative growth rate, and identify robust QTL. We use the Lemnatec imaging platform of The Plant Accelerator to measure the growth of Gladius/Drysdale recombinant inbred lines under well watered and drought conditions. Using a 9k Illumina SNP map, QTL were identified for growth rate, transpiration, water use efficiency in both conditions. We also identified QTL for yield and yield components in a drought experiment conducted in semi-controlled field conditions on the same population. We found co-located QTL for plant growth and yield components indicating that imaging platform could be used to phenotype recombinants lines for positional cloning of target QTL. To our knowledge this paper is the first study applying automatic imaging platform to genetic population to identify QTL for growth response to drought in wheat.

Environmental chacterization and QTL detection to dissect wheat tolerance to drought and high temperature

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Wheat is the most widely cultivated crop worldwide and it experiences a wide range of stresses including drought and high temperature. Identifying traits and chromosomal regions linked to such stress is then of paramount importance. The aims on this work were first to precisely characterize the environments to determine the nature, timing and relative intensity of stresses experienced by the plants, secondly to dissect the genotype x environment interaction (GEI) to understand genotypes performances across environments, and thirdly to identify QTL involved in tolerance to those stress.

Three bread wheat recombinant inbred lines populations created with elite lines combining complementary tolerance to drought and high temperature stresses were grown over three years under irrigated and drought winter sowings, and heat-irrigated spring sowing in the north-western Mexican desert of Sonora.

An environmental characterization was performed per trial. Each crop cycle was divided into three development stages. Stress thresholds were established and used on relevant environmental factors to get environmental parameters (EP) values. A clustering step on this dataset allowed identifying eight clusters of parameters. From each cluster, a representative EP called medoid was extracted. Such reduced dataset represented 99.7% of the environmental variance. A principal component analysis (PCA) on eight medoids discriminated environments and identified stress characteristics experienced by plants which PCA of agronomical data did not achieve. Multiple regression models to explain agronomical traits with medoids displayed R^2 ranging from 0.82 to 0.97, with a drought and then a heat cluster usually involved. Stresses occurring during the grain set phase caused major impact on grain yield.

GEI were then studied using medoids as covariates. Yield and associated traits were used whatever the condition. A highly significant GEI variance was observed for the tested traits. EP enabled to dissect and explain part of the interaction. To finish with, constitutive and environment specific QTL were detected in the three mapping populations.

The methods and results obtained will help understanding how plants interact with their environments and the tolerance mechanisms involved. After validation through fine mapping they could be used to improve European wheat drought and heat tolerance, by pyramiding interesting QTL.

Genetic analysis of heat tolerance components in wheat

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Heat episodes during grain filling significantly impact wheat yields worldwide. As a first step in identifying chromosome regions controlling heat tolerance that might be useful in breeding, an F_1-derived doubled haploid population (DH) of 144 lines, made by crossing the two Australian wheat varieties, Drysdale and Waagan, was evaluated for heat responses in greenhouse/chamber experiments conducted at two times of the year. The 9,000-feature wheat SNP array was used to construct a molecular genetic map comprised of 550 unique genetic loci, spaced on average 4.8 cM apart. Plants in both trials were exposed to a brief heat stress (3 days at 37/27°C day/night), 10 days after anthesis, and then scored for developmental, architectural, senescence and yield component traits. The heat treatment reduced final grain weight and grain filling duration, shoot biomass (stem + leaves), and accelerated chlorophyll loss. In both trials, only a single QTL (on 3BS) was detected for the grain weight response, explaining 12-20% of the phenotypic variation for that trait, and with Waagan contributing the tolerance allele. It was also the dominant QTL for senescence responses, with stay-green being positively associated with grain-weight maintenance under heat. In non-stressed plants, the QTL on 3BS was one of several influencing chlorophyll content per se, with the Waagan allele on 3BS conferring higher chlorophyll content. The 3BS QTL also affected shoot biomass under heat conditions. The results of this study will be discussed in relation to potential heat tolerance mechanisms and trait associations observed in a trial of a germplasm panel performed under similar heat stress conditions.

Genetic analysis of tolerance to salt stress in wheat using association mapping

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Wheat, the most important cereal crops and the main stable food in most parts of the world, is adversely affected by soil salinity leading to low yields. How to improve the crops salt tolerant and its adaptation to the increasing soil salinity has become of paramount important to Wheat breeders. The early stage salt tolerance in wheat has been considered as a major determinant of its stable establishment in saline soil. To identify QTL that confers salt tolerance in wheat at the early stage, we carried out a genome-wide association study (GWAS) using 150 unrelated wheat germplasm genotyped with 90,000 SNPs Illumina iSelect SNP assay. The responses of the studied germplasm to salt stress and salt types (*i.e.*, NaCl and Na_2SO_4) at the early stage of growth were evaluated in the controlled and/or greenhouse conditions. Traits such as the seed germination potential, root length, shoot length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight and seedling survival percentage were collected. We generated the salt tolerance indices for each genotype as a ratio of the measured trait in salt treatment and in control. Data collected were spatially analyzed by linear mixed models using the residual maximum likelihood (REML) routine in GENSTAT, due to the evidence of positional effects in the experimental design. We used Wald statistics to assess the significance of the main effects and their interactions and, the predicted means values were used in the GWAS analysis. Results revealed the existence of high variation among the genotypes. All the traits except root length (H_b: 8%) showed high broad sense heritability values ranging from 61.8% to 90%. Thus, giving credence to the quality of phenotypic data which would allow for the identification of QTL linked to salinity tolerance with substantial power. Among the computed salt tolerance selection indices, dry shoot weight was highly positively correlated with shoot length, fresh root weight, fresh shoot weight and dry root weight salt tolerance selection indices. These traits can be dependently selected as measures of salt tolerance.

Genome-wide transcriptome analysis of Pi-stressed wheat seedlings

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Plants have developed several morphological and physiological strategies to adapt to various environmental stresses. Although the molecular mechanisms for the response and acclimation of plants to various stresses are complex, better understanding of them are important and useful not only for the basic science but also for the applied works such as new variety breeding. Transcriptome analysis using NGS technology provides high-resolution data and is a powerful tool for studying global transcriptional networks. Therefore, we have performed RNA-Seq to analyze various abiotic stresses of rice, in which sequence reads were mapped to the reference genome sequence.

In this study, we applied RNA-Seq technique to analyze phosphate starvation (–P) stress of wheat. The reference genome sequence and gene annotation of wheat are still incomplete, so the strategy that we used in rice is not directly applicable to wheat. Therefore, at first we constructed a transcript dataset containing 29,617 non-redundant transcripts, comprising 14,570 full-length cDNAs from the TriFLDB database and 15,047 contigs produced by de novo assembly analysis (available from Komugi GSP web site, http://komugigsp.dna.affrc.go.jp/index.html). We identified 892–2,833 responsive transcripts in roots and shoots under –P from the sequence reads mapped to our newly constructed transcript dataset. The comparative analysis of the wheat- and rice-responsive transcripts (Oono et al. 2011 Rice) under –P specified upregulated transcripts common to both plants. Most of them appear to be involved in the general response to –P, that is the IPS1-mediated signaling cascade and its downstream functions such as Pi remobilization, Pi uptake, and changes in Pi metabolism (Oono et al., 2013 BMC Genomics).

The comparative transcriptome analysis between wheat and rice is thought to be a powerful tool to identify the important adaptation mechanisms to other stresses than the –P stress and it can accelerate the functional genomics studies in wheat. The wheat transcript dataset in this study as well as our RNA-Seq data in rice is useful as valuable resources for wheat genomics and genetics.

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Tiller number in barley is influenced by ABA

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Cultivated barley, derived from its wild progenitor *Hordeum vulgare* ssp. *spontaneum*, is among the world's earliest domesticated crop species and today represents the fourth most abundant cereal in both area and tonnage harvested (http://faostat.fao.org). Approximately three-quarters of global production is used for animal feed, 20% is malted for use in alcoholic and non-alcoholic beverages, and 5% as an ingredient in a range of food products. So improving barley crop yield is one of the most important challenges for research scientists, based on its food and commercial values.

Barley plant architecture is crucial for grain yield and is determined by plant height, tiller number, grain number, grain size, and spike size and spikelet number in a spike, associated with barley productivity. The tiller number in barley is an important agronomic trait for grain production. However, the molecular mechanisms for generating tillers and increasing yield potential remain to be elucidated.

The plant hormone abscisic acid (ABA) mediates plant responses to different kinds of abiotic stress such as drought stress and is involved in long-distance signaling in plants. It is the key signal regulating stomatal aperture, seed development, embryo maturation, synthesis of storage products (proteins and lipids), desiccation tolerance and is involved in apoptosis and maintenance of dormancy (inhibition of germination). In concert with other plant signaling molecules, ABA is also implicated in mediating responses to pathogens and wounding. Certainly, ABA homoeostasis in the plant is tightly controlled by a balance between biosynthesis, inactivation and degradation. But its function in tiller development has been unknown.

In order to increase drought resistance two independent transgenic lines (LOHi236, LOHi272) have been generated that are posttranscriptionally silenced for *ABA 8'-hydroxylase* genes (RNAi). The transgenic lines showed more tillers resulting in increased yield under control conditions. It was found that during development a temporal increase in ABA content in the late vegetative phase occurs. The followed decrease of ABA content is affected in the two transgenic lines, correlating with the extended phase of active tiller outgrowth.

Identification of favorable alleles in wild barley for waterlogging tolerance

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Agriculture is one of the societal sectors likely to be most strongly affected by climate change. Present climate projections predict temperature increases, which will lead to elevated CO_2 in the atmosphere and prolonged growing season in northern latitudes. Elevated CO_2 is estimated to enhance photosynthesis and thereby increase crop yield. However, higher precipitation, especially in spring and fall, and frequent periods of intense rainfall are expected in northern Europe. This will lead to flooding and waterlogging, and O_2 deficiency in the soil, resulting in reduced yield. Wheat and barley are among the most important crops in Europe. They are sensitive to waterlogging and the production may be reduced up to 40% depending on cultivar. Thus, to make use of a longer growing season and elevated CO_2 the cultivars have to be adapted to climate change conditions.

This project focuses on pre-breeding for waterlogging tolerance in barley. Our aims are to *i)* identify waterlogging-tolerant genotypes among accessions of wild barley (*Hordeum spontaneum*), Nordic landraces and modern cultivars and *ii*) identify novel quantitative trait loci (QTL) and genes associated with waterlogging tolerance for transfer of tolerant genes into northern barley cultivars. We have compared the performance of 28 different *Hordeum* accessions under waterlogged and normal water conditions studying leaf and tiller number, percentage of chlorotic leaves, SPAD meter measurement of the chlorophyll content of the first five leaves and shoot dry weight at different time points. Most studied landraces were highly susceptible, while tolerant accessions were found among both cultivars and wild barley. Based on the large variation found among these accessions we identified suitable parents for generating QTL mapping populations. In a doubled-haploid mapping population derived from a cross between a susceptible cultivar and a tolerant wild barley accession we identified several QTL with large effect and favorable wild barley alleles for waterlogging tolerance. These QTL will be verified by genetic marker and phenotype association observed in near-isogenic lines (wild barley segments in a cultivated barley genetic background). Our results suggest that wild barley is an important genetic resource for improving waterlogging tolerance in modern barley cultivars.

Session IV

Biotic Stress Resistance

Harnessing resistance to biotic stress in cereals – past and future

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Barley and wheat are of special importance for feeding the earth´s growing population. However, both are hit by many pathogens causing severe yield losses. Therefore, identifying sources of resistance followed by marker development and the marker based exploitation of genetic and/or allelic variation with respect to resistance is a prerequisite to ensure an ecological sound cereal production and to avoid high yield losses. In the last decades molecular markers have been developed for many major genes and QTLs for resistance in wheat and barley and turned out to be efficient tools in different marker based selection procedures, e.g. marker assisted backcrossing or pyramiding of resistance genes. While in the past marker development was time consuming and laborious, today genomic resources like the Infinium iSelect genotyping bead chips, physical and sequence based maps, the GenomeZipper, comprising a virtual linear order of genes of different monocot species, next generation sequencing techniques, e.g. genotyping by sequencing (GBS), exome capture, or RNAseq and MACE, facilitate efficient marker development and saturation for genes and QTL of interest, as well as expression analysis, even in wild relatives like *Hordeum bulbosum* or *Triticum monococcum*. This allows the marker based incorporation of resistance genes or QTL derived from genetic resources with a minimal linkage drag and considerably enhances marker saturation for gene isolation via map based cloning. Using these genomic resources resulted recently in the isolation of the BaMMV/BaYMV resistance gene *rym11,* the identification of genes involved in pre-haustorial resistance of *T. monococcum* against *Puccinia triticina,* and is at present applied to several major genes and QTL encoding resistance to viral (BaMMV/BaYMV, BYDV) and fungal pathogens (*Puccinia hordei, Blumeria graminis*). The isolation of genes involved in resistance will transfer resistance breeding to the allele level and will facilitate the sequenced based identification of novel alleles in large gene bank collections and their directed use in plant breeding. In summary, respective genomic tools will lead to a faster exploitation of the genetic diversity present with respect to resistances in the wheat and barley genepool, which is a prerequisite to cope with future challenges, e.g. caused by climate change.

Suppression among alleles of an NB-LRR resistance gene interferes with resistance in F_1 hybrids and allele-pyramided wheat plants

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In plants, immune receptors of the NB-LRR class mediate race-specific resistance against pathogen attack. This type of resistance is often rapidly overcome by newly adapted pathogen races when employed in agriculture. The stacking of different resistance genes or alleles in F_1 hybrids or in pyramided lines is proposed as a strategy for more durable resistance. Here the pairwise combination of different alleles of the powdery-mildew-resistance gene *Pm3* in F_1 hybrid and double-transgenic wheat lines is reported. We show that a suppression activity among the *Pm3* alleles interferes with resistance stacking and we demonstrate that the suppression takes place at the posttranslational level. Using a transient-expression system in *Nicotiana benthamiana*, the LRR domain was identified as the suppression-conferring domain. The results of this study suggest that the expression of closely related NB-LRR resistance genes or alleles in the same genotype can lead to dominant-negative interactions, which is a molecular explanation for and a first step to overcome the frequently observed ineffectiveness of resistance genes introduced from the secondary gene pool into polyploid crop species.

Fusarium head blight of oats: Infection, germination and resistance

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Fusarium head blight is a major concern of food and feed growers, processors and consumers due to the associated mycotoxins. The disease affects wheat, barley and oats. Oats are generally considered more resistant to FHB than wheat but significant amounts of *Fusarium* toxins are being detected in oats from Norway. In this abstract, results from our studies on the infection process of *Fusarium* spp. and its effect on DON and germination of oats and identification of QTLs for *Fusarium* resistance are presented.

Fusarium graminearum entered primarily through the floret mouth into the floret cavity. Both visual symptoms and fungal infections started at the apical portions. Basal portions of the floret were found diseased under severe infection and the whole floret was colonized. Profuse hyphal growth was observed on the anthers. Disease development within the panicle was slow and was primarily by physical contact between adjoining florets. Oats were most susceptible for *Fusarium* infection at flowering. This was displayed by highest DON and seed infection levels along with lowest germination percentage recorded at inoculations done at flowering as compared to later inoculations. Early infections killed the developing seed, and late inoculations reduced germination through seedling blight. Seed germination was often far below seed viability, and a combination of seed dressing and dehulling restored germination to the percentage of viable seeds. Correlation coefficients between germination and DON level of samples from spawn- and spray- inoculated oat nurseries varied between - 0.412 and -0.711. In addition to DON level, seed infection affected germination of *Fusarium*- damaged oats, especially in conditions that facilitate late infections (*1, 2*).

Two recombinant-inbred line populations, Hurdal x Z595-7 (HZ595, with 184 lines) and Hurdal x Z615-4 (HZ615, with 91 lines), were used for QTL mapping and were phenotyped for DON content, FHB severity, plant height and days to heading and maturity. A QTL for DON on chromosome 17A, designated as *Qdon.umb-17A*, was detected in all experiments using composite interval mapping, with phenotypic effects of 12.4-27.9% and the resistance allele from Hurdal. In addition, QTL for DON were found on chromosomes 5C, 9D, 13A, 14D and unknown_3 in HZ595 and 7C in HZ615. QTL for FHB were found on 11A in HZ595 and 13A_3 and unkown_7 in HZ615, several of which coincided with QTL for DH, DM and/or PH. Three SNPs closely linked to *Qdon.umb-17A* were identified in both populations (*3*). To use these SNPs in marker-assisted selection seems limited by the lack of markers in Nordic germplasm, which went through a severe bottleneck about 100 years ago(*4*). Still progress is being made in reducing DON contents. However, a wider genetic variation and more markers are needed.

Genomics-assisted cereal pre-breeding for pest and diseases for global food and bio-security

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Pest and diseases are globally responsible for the 25-30% of pre-and post harvest crop losses. With the increasing demand for food due to increasing world population and lack of food where it is most needed human popualtions living in particularly developing countries are at serious risk. Global engagement is necessary to share knowledge and genetic resources to fend off eminent food shortage and prevent biosecurity problems.

Pre-breeding research has a significant role in linking between genetic resources and plant breeding as it shortens the time frame between genetic enhancement and the development of new, improved crop varieties with higher quality and yields. With the ever decreasing cost of DNA sequencing a wide range of genomic solutions and tools such as highly dense genetic maps with SSR, DArT, and GBS-based SNP markers are available for breeding new crop varieties that are high yielding and resistant to pest and diseases.

Our recent focus has been on identifying key genetic resources and relevant genes for pest and diseases such as Russian wheat aphid (RWA), Sunn pest, Karnal bunt and Stripe rust. These insects and diseases are most damaging pests of cereals in many cereal growing countries and they cause substantial yield loses globally.

We have identified new sources for all the pests, and mapped new genes (using SSR, DArT, and GBS-based SNP markers) with broad resistance against RWA biotypes present in at least seven countries. 1D and 7D chromosomes were found to harbour multiple resistance genes (gene complexes) for RWA. In barley, apart from the previously reported genes, we have identified new genes on chromosomes 5H and 7H, providing resistance to highly virulent aphid biotypes. We have mapped genes for Karnal bunt and stripe rust from both Australian and Indian wheat varieties. These pests present biosecurity risks to many countries and breeding resistance against them should prevent future crop losses. The presentation will also outline the strategies and the current progress for gene introgressions to adapted varieties, and provide perspectives for future developments.

Association mapping in European winter wheat

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Results from a long term study (WHEAT, 2008-2011 and VALID, 2011-2014) regarding genome-wide association mapping in European winter wheat will be presented. A comprehensive dataset based on multi-environmental field data was developed for yield, agronomic traits, baking quality and resistance to fungal diseases. The genotypic data consisted of 770 genome-wide microsatellite loci and a set of 7934 Illumina iSelect Infinium SNP markers.

As an example for a comprehensive association analysis we present results on resistance to three fungal diseases, i.e. *Fusarium* head blight caused by *Fusarium graminearum* and *Fusarium culmorum*, *Septoria tritici* blotch caused by *Mycosphaerella graminicola* and tan spot caused by *Pyrenophora tritici-repentis*. The phenotypic data were generated in replicated field trials at different locations and over several years respectively, using a panel of 358 European winter wheat and 14 spring wheat varieties. The analysis of marker-trait associations was carried out by using a mixed linear model and a kinship matrix for population stratification. We will summarize the analysis of marker-trait associations and the implications for plant breeding.

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Genome-wide association scan for stripe rust (*Puccinia striiformis* f. sp. *tritici*) response in spring hexaploid wheat (*Triticum aestivum* L.) from worldwide

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Stripe (yellow) rust of wheat, caused by *Puccinia striiformis* f.sp. *tritici* (PST), is an ubiquitous, polycyclic wheat disease capable of long-distance migrations and rapid evolution of host-pathogen interaction mechanisms. The major negative impact on wheat production stimulates a continuous search for effective resistance genes, either belonging to the race-specific all-stages expressed or to the adult partial resistance classes. In this study, one thousand spring wheat accessions of the USDA-ARS National Small Grain Collection from 89 countries worldwide were characterized for field PST response (infection type, IT and severity, SEV) under 6 US Pacific Northwest environments (Washington and California) and for response to four single PST races at the seedling stage. Genotyping was performed with the Infinium wheat SNP 9K platform. Population genetic structure was assessed through Ward clustering and a model-based Bayesian clustering method (STRUCTURE). The analysis pointed out the presence of four main population groups and seven sub-groups that reflected in part the geographic origin. Linkage disequilibrium among SNPs fallen below the reference r^2 values of 0.8, 0.5 and 0.3 at 0.002, 0.01 and 1.6 genetic distances (cM), respectively. Field response heritability ranged from 0.72 to 0.87 and reached 0.89 (IT) and 0.91 (SEV) across environments. Population structure showed a moderate effect on PST responses with R^2 values equal to 13.8% (IT) and 15.5% (SEV) for the combined data across environments. The GWAS conducted using a mixed linear model including the kinship matrix among accessions allowed us to identify 73 loci (identified by single or multiple associated SNPs) significantly associated to PST response in three or more environments, after excluding heading and/or plant height effects. Twelve loci showed a preferentially association to SEV as compared to IT. Thirty-eight out of the 73 loci showed concomitant effects to one or more of the PST races. Chromosome positions of loci were compared to those of known *Yr* genes and QTLs based on relative chromosome distances. Considering accessions with moderate to susceptible ITs only allowed us to map 34 loci preferentially associated to partial resistance. The results provide a base for more efficient exploitation of germplasm resources for PST-resistance genomic-assisted breeding.

Powdery mildew resistance gene *Pm8* derived from rye is suppressed by its wheat ortholog *Pm3*

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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.) chromosomes into wheat cultivars. The most widely used introgression nowadays is the 1BL.1RS translocation derived from the rye cultivar Petkus carrying amongst three rust resistance genes the powdery mildew resistance gene *Pm8*. *Pm8* has recently been cloned and shown to be the rye ortholog of the *Pm3* wheat powdery mildew resistance gene on wheat chromosome 1AS. Both genes code for coiled-coil (CC), nucleotide-binding site, ARC1 and ARC2 (NB-ARC) and leucine-rich-repeat (LRR) domain proteins and mediate race-specific resistance against powdery mildew in wheat. However, not all wheat lines carrying the translocation show resistance to *Pm8* avirulent isolates due to the presence of a suppressor gene in wheat. Interestingly, this suppressor gene was mapped to a region encompassing the *Pm3* locus in wheat. In a single-cell transient expression assay we found that the *Pm3* gene is sufficient to suppress *Pm8*-mediated resistance. This result was further confirmed in crosses of transgenic lines carrying, besides the *Pm8* transgene, a *Pm3* transgene. Such lines showed isolate dependent suppression of *Pm8* while the sister lines only carrying *Pm8* were completely resistant. Expression analysis revealed that suppression is not the result of gene silencing but due to post-translational processes most likely involving protein interactions. Thus, our results give a first molecular explanation for a frequently observed suppression phenomenon when chromosomes from wheats with lower ploidy level and wild relatives are introgressed into wheat.

Screening for *Sipha maydis* resistance in Argentinean commercial varieties

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Sipha maydis (Passerini) is a new aphid pest of cereals and cultivated and wild grasses that was recently introduced into America. This species was first reported in Argentina in 2002 in Mendoza province, close to the Andes and currently it is widespread in the main cereal producing area. This aphid damages mature cereal plants in late spring by reducing leaf area and inhibiting ear expansion. The young leaves of infested wheat become chlorotic, which results in reduced plant growth and flag leaf expansion with consequent decrease in yield. Since there are none previous report of resistance against this new pest in Argentinean bread wheat varieties, the aim of this research was to assess the defence strategies of 47 commercial cultivars. The antixenosis, antibiosis and tolerance mechanisms of plant resistance were determined by traditional tests. It was used a population of *S. maydis*, collected from 2004 to 2010, with a wide genetic variability. Antixenotic type of resistance was identified in 24% of the cultivars. Only four varieties, with the same genetic background, showed a high antibiotic level that did not allow the aphid survivorship. One half of the cultivars had an intermediate level of tolerance and the rest showed significant losses in aerial biomass, dry weight and chlorophyll contents, when subjected to infestation. The scarce resistance found in commercial cultivars and the wide range of climatic conditions this species is adapted to is likely to make the control of this pest very difficult. An adequate level of resistance was identified in only a few varieties, their use would enable the breeding of new wheat lines by crossing with genotypes carrying other desirable genes affecting the aphid cycle, and/or conferring tolerance to *S. maydis* to broaden aphid resistance breeding.

Linkage disequilibrium and association analysis of stripe rust resistance in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) population in Israel

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Genome-wide association mapping (GWAM) is becoming an important tool for the discovery and mapping of loci underlying trait variation in crops, but in the wild relatives of crops the use of GWAM has been limited. Critical factors for the use of GWAM are the levels of linkage disequilibrium (LD) and genetic diversity in the target populations, particularly in those of self-pollinating species. Here we report LD estimation in a population of 129 accessions of self-pollinating wild emmer, *Triticum turgidum* ssp. *dicoccoides*, the progenitor of cultivated wheat, collected in Israel. LD decayed fast along wild emmer chromosomes and reached the background level within 1cM. We employed GWAM for the discovery and mapping of genes for resistance to three isolates of *Puccinia striiformis*, the causing agent of wheat stripe rust. The wild emmer population was genotyped with the wheat iSelect assay including 9,000 gene-associated SNP markers (wheat 9K Infinium) of which 2,278 were polymorphic. The significance of association between stripe rust resistance and each of the polymorphic SNP was tested using mixed linear model implemented in EMMA software. The model produced satisfactory results and uncovered two significant associations. One in a well known cluster of stripe rust resistance genes on the chromosomal arm of 1BS and one on 1BL where no significant stripe rust resistance genes are known. Successful discovery and mapping of stripe rust resistance genes indicated that GWAM can be used for gene discovery and mapping in wild emmer. The developed resource provides an opportunity to perform the genetic dissection of agronomic traits in wheat at high resolution and facilitates the utilization of ancestral populations in wheat breeding programs.

Towards deciphering a novel barley yellow dwarf virus (BYDV) tolerance introgressed from *Hordeum bulbosum* by the use of genomic resources and Next-Generation-Sequencing (RNASeq and exome capture)

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Hordeum bulbosum has been shown to be a valuable source for introgressing new resistances into cultivated barley (*H. vulgare*). Due to climate change Barley yellow dwarf (BYD), which is the most important virus disease of cereals worldwide, has become increasingly important in Germany. A set of *H. bulbosum* introgression lines has been screened for tolerance using a BYDV-PAV isolate. A barley line carrying a *H. bulbosum* introgression on chromosome 2HL turned out to be highly tolerant and DH-lines derived from the backcross (Emir x *H. bulbosum*) x Emir have been analysed in five steps in order to minimize the size of the BYDV carrying fragment. Firstly, out of 221 DH-lines 27 plants carrying a recombination event in the *H. bulbosum* fragment were selected based on nine PCR markers and at the Illumina BeadXpress Array (384 SNPs). The size of the *H. bulbosum* introgression was estimated to be about 25 cM. Next, analysis on the 9k iSelect bead chip revealed about 50 polymorphic SNP markers and allowed narrowing down the introgression carrying the BYDV-tolerance to about 3 cM. In a third step, the region harbouring the BYDV-tolerance was further delimited by the exploitation of information provided by the barley genome zipper. A set of 13 markers derived from the genome zipper was successfully mapped within the target interval and the map was anchored to the barley physical and sequence map. Furthermore, based on phenotypic data and respective markers, the tolerant *H. bulbosum* introgression line and the susceptible parent Emir were subjected to RNAseq profiling after virus infection and revealed 12 down regulated genes located on chromosome 2HL. In a fifth step exome capture was conducted and allowed further marker saturation and identification of genes involved in virus tolerance. Additionally, a non gridded BAC library was constructed from the tolerant line towards the construction of a physical map for the telomeric region carrying the tolerance locus and validation of candidate genes. Results obtained up to now revealed the presence of a novel BYDV tolerance derived from *H. bulbosum* and provided the tools for the efficient deployment of this tolerance in barley breeding.

Session V

Quality for Food and Industrial Use

Cereal quality – time for a paradigm shift?

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Advances in agronomy, crop nutrition, protection and breeding led to a bounty in crop productivity in the second half of the 20th century in the Western world. Industrial agriculture led to reasonable food prices and control of starvation in developed countries, but it also brought various environmental issues. With respect to nutrition, the consumption of large amounts of calories combined with a lifestyle with no or irregular physical activity has led to the prevalence of obesity and its associated health concerns.

In addition, a monotonous cereal based diet has caused micro-nutrient deficiencies especially in developing countries. Currently, new quality traits such as vitamin, phytonutrient and mineral content and their nutritional benefits are promoted by nutritionists and are in increasing demand by health orientated consumers. Novel processing technologies enable the exploitation of bioactive bran compounds in breads with high volume and soft texture. Therefore, cereal breeders have to be ready to re-define their breeding targets with respect to quality and be willing to partner with end users (processors and consumers) to create high value genotypes for commercialisation. We present case studies of recent innovations of bio-fortified wheat.

Besides food, the demand for specific qualities of cereal crops is also growing with respect to feed and fuel. Barley, despite its recent use as a specialty food crop, is still a major feed grain. Single mutations of barley such as the *rob1* (orange lemma – low lignin) or *Kap1* (hooded lemma – no awn) gene have great potential for exploitation in the development of dual purpose varieties which can tolerate grazing and recover to produce grain or provide forage, silage or hay. Moreover, cereal straw of low lignin mutants represents a resource for sustainable bio-materials and biofuels.

Quality traits relevant for human nutrition in European oat genetic resources collections

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Oat is a crop with an important European history and tradition. It has still high breeding potential, based on a wealth of genetic resources represented in genebank collections. Its high value in human nutrition is unique among cereals and widely recognized. It is based on high contents of extractable dietary fiber, including β-glucan, with documented effects on blood glucose and cholesterol levels. Currently phenolic compounds with antioxidant and anti-inflammatory effects receive increasing interest in nutrition science. Oat is also rich in protein and fat. With a targeted action funded by EU DG AGRI based on regulation EC 870/2004 a broad spectrum of European oat genetic resources representing wild *Avena* species, landraces, traditional, obsolete and modern cultivars, was observed in a multi-location field study all over Europe. Dehulled groats were analyzed for traits relevant to quality in human nutrition.

Genotype, environment and interaction effects, as observed on a set of standard cultivars, were highly significant for most of the targeted traits. The dominating fibre fraction is non-starch polysaccharides (NSP). Lignin and uronic acids are minor fractions. NSP ranged from 5.3-14.1% in hexaploid cultivated oats. Up to 15% were found in wild *Avena* species. Total and soluble ß-glucan, the nutritionally most important parts of NSP, ranged from 2.2–6.8% and 1.2–5.3%, respectively. The broadest range was found in the traditional cultivars. Diploid and tetraploid marginally cultivated and wild species tended to be higher on average, but rarely exceed the range of hexaploid cultivated oats. Singular contents up to 6.3 and 6.6% total ß-glucan were found for local material collected in Greece and Moldavia. Magda, a cultivar of naked oats, significantly outperformed the best standard cultivars in NSP, total and soluble ß-glucan. Diploid wild species reached more than 5% soluble ß-glucan.

Of the antioxidants higher contents than previously reported in oats were frequently found. High values of α-tocotrienol and avenanthramides were expressed by *A. strigosa*. It was not dominating in the group of accessions with high α-tocopherol content. Cultivars of *A. sativa* reached up to 100 mg kg^{-1} α-tocotrienol and 65 mg kg^{-1} α-tocopherol. Contents of ß, γ- and δ-tocols were low.

Purple-grained wheat: Genetic control, molecular mechanisms and practical value

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The gene *Pp3* (purple pericarp) mapped to wheat chromosome 2A (in *Triticum durum* and *T. aestivum*) has been isolated and shown to encode MYC-like regulatory factor. For activation of the anthocyanin biosynthesis the complementary action of the *Pp3* with another gene, *Pp-1* (homoeologous group 7 chromosomes), encoding MYB-like regulatory factor, is needed. To clarify regulatory mechanisms of anthocyanin biosynthesis in wheat pericarp we obtained *T. aestivum* near isogenic lines carrying different combinations of *Pp* alleles, using marker-assisted backcrossing approach. Quantitative analysis of transcription of the anthocyanin biosynthesis structural genes and the regulatory *Myc1* gene in pericarp of these lines was performed. For activation of some structural genes (i.e. gene encoding chalcone-flavanone isomerase) the presence of the dominant allele of one gene was sufficient, whereas, flavanone 3-hydroxylase (F3H) expressed in the presence of dominant alleles of the both complementary genes, suggesting the *F3h* gene activation to be the main checkpoint in the anthocyanin biosynthesis regulation. Some suppressive effect of the dominant *Pp-1* allele on transcription of *Pp3* (*Myc1*) was found.
Practical value of purple pericarp colour is usually discussed in the literature in relation with antioxidant capacity of anthocyanins and hence with usefulness of purple-grained wheat for human nutrition. However, results obtained in our studies suggest broader potential of this trait for practical value, in particular related with seedlings drought tolerance and seed longevity. Better longevity of seeds in the presence of anthocyanins was observed in barley as well. The possible mechanisms are discussed.

The complex regulation of wheat grain storage protein synthesis unraveled through GWAS

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The end-use value of bread wheat (*Triticum aestivum* L.) grain is mainly determined by grain storage protein (GSP) content and composition. In wheat, the two main classes of GSP are glutenins, composed of high-molecular-weight (HMW-GS) and low-molecular-weight (LMW-GS) subunits, and gliadins, composed of four classes (ω1.2, ω5, α/β and γ). GSP are encoded by genes mainly controlled at the transcriptional level by a network involving at least eight transcription factors (TFs). This transcriptional network could lead to the stable allometric scaling relationships observed between the quantity of gliadins or glutenins and the total quantity of nitrogen per grain (N_{tot}). Chromosomic regions involved in the regulation of GSP synthesis were identified using linkage mapping in 196 accessions of a bread wheat worldwide core collection grown in three environments in France. We used 873 markers for genome-wide association and 167 single nucleotide polymorphism markers in 51 candidate genes, including 35 TFs expressed in the grain, for candidate association. Associations between genotypes and phenotypes were tested using the general linear model. The structure of the collection used was taken into account to avoid false negatives. We identified 95 associated loci, 29 (31%) of which were inside or in strong linkage disequilibrium with candidate genes. As expected, structural GSP genes were associated with studied traits. In addition, several loci putatively trans-regulating GSP accumulation were uncovered. Seven candidate TFs were associated or in strong linkage disequilibrium with markers associated with the composition or quantity of glutenin or gliadin, or allometric grain N allocation parameters, confirming the importance of the transcriptional control of GSP accumulation. Two loci on 3DL and 7DS were strongly associated with the LMW-GS to HMW-GS ratio, the percentages of GSP and glutenin in N_{tot}, and the scaling coefficient of the glutenin-N_{tot} relationship. For gliadins, a chromosomic region on 6BS affected the proportion of gliadin classes in total GSP or gliadin while the proportion of total gliadin in N_{tot} was controlled by marker cfd43 on 2DS. Thus, our results suggest that the genetic determinants of glutenin and gliadin compositions are mostly distinct from each other.

A TILLING strategy to improve the nutritional health value of wheat

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Diet and lifestyle are considered the main causes for the occurrence of several chronic diseases, including diabetes, obesity, dyslipidemia, hypertension and cardiovascular disorders.

Starch, the major component of wheat grain, is composed of two glucan polymers, amylose and amylopectin. The relative amount of these polymers is the main parameter associated with starch functionality at nutritional and technological level. Nutritionists have a growing interest in resistant starch (RS) that is correlated with the amount of amylose in the kernel. Overall there is substantial evidence that RS possesses a wide range of benefits for human health, playing a role similar to dietary fibre.

We have used a TILLING (Targeting Induced Local Lesions IN Genomes) approach to improve the nutritional value of flour and semolina by means of the production of novel high amylose wheat genotypes. Genes encoding the starch branching enzymes IIa (*SBEIIa*) have been targeted in two EMS-mutagenised populations deriving from the bread wheat cv Cadenza and the durum wheat cv Svevo, respectively. This analysis resulted in the identification of 123 mutations in the *SBEIIa* homoeoalleles in bread wheat and 45 in durum wheat.

Pyramiding of different single *null* homoeologous allowed us to produce complete *null SBEIIa* mutants, that showed a strong increase of amylose content and RS compared to cv Cadenza and Svevo.

Identification of functional mutations in arabinoxylan synthetic genes in hexaploid wheat

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The polysaccharide arabinoxylan is the main constituent of wheat endosperm cell walls and typically constitutes about 2% (w/w) of white flour. It has an important role as part of dietary fibre in the human diet, but has a negative influence on non-food uses of wheat such as fermentation and animal feed. We have recently shown by RNAi suppression that the main wheat genes responsible for AX backbone synthesis in starchy endosperm are TaGT43_2 and TaGT47_2 (Lovegrove et al., 2013 Plant Physiol. 163: 95-107) and that TaXAT1 is responsible for mono-substitution of xylose by arabinose in AX (Anders et al., 2012 PNAS 109: 989-993). The RNAi construct specifically repressed expression in the endosperm but mutations in these genes will have constitutive effects. Since TaGT43_2 and TaXAT1 expression is mostly confined to endosperm, these genes were selected as the best targets for mutations which are unlikely to affect other tissues. Here we describe the identification of plants carrying mutations in three homoeologues from the A, B and D sub-genomes for both genes in an EMS-mutagenised hexaploid wheat population using the methodology previously described (Botticella et al., 2011 BMC Plant Biol. 11, 156). We have combined some mutations in homoeologues of the same gene into one plant by crossing. We have selected plants which are homozygous for single or double mutations for phenotypic analyses of AX in mature endosperm. We will present the results of analysing the amount and structure of AX of these homozygous plants. By comparison of results with those from RNAi plants, we will seek to separate effects of transgene expression from the roles of particular encoded proteins in synthesising different fractions of AX. By comparison of relative effects of mutations in different homoeologues and combinations of homoeologues, we intend to assess the extent to which the effects of the mutations are additive, and whether there is evidence of compensation from other homoeologues in response to a functional mutation in one homoeologue.

Screening for speltoid off-types in bread wheat – Discrimination of alleles and copy numbers at the domestication gene Q using quantitative pyrosequencing

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Undesirable speltoid bread wheat spikes can be the main reason for increased heterogeneity and consequently rejection of a wheat cultivar candidate. Pyramidal spikes with an elongated rachis and features similar to wild grasses such as tenacious glumes are characteristic for a speltoid phenotype. Plants with two copies of the domestication gene *Q* on the long arm of wheat chromosome 5A express a normal square headed spike morphology. Copy numbers lesser or more than two lead to speltoid or compact spike shapes, respectively. A quantitative pyrosequencing assay (Förster et al. 2011) was developed to distinguish between normal wheat plants with two copies of the *Q* allele and aberrants. These aberrants can be aneuploids either with a reduced number of chromosome 5A copies or plants which carry the primitive 5A*q* allele. A reproducible determination of the *Q* gene copy number is now possible, based on homoeologous peak height quantification, from a pyrosequencing-based pyrogram. The calculated *Q* ratio (*5AQ*/*5Bq* and *5Dq*) allows to quantify copy number variation at the *5AQ* locus.

Based on 10 licensed German wheat varieties, 371 single progeny plants originating from speltoids were molecularly and phenotypically characterized in 2011 (Förster et al. 2013). A 98.06% correspondence between spike morphology and the *Q* gene copy number estimation using pyrosequencing indicates that this method is highly reliable and suitable for high throughput screening. In future, a quantitative pyrosequencing assay may be applied in wheat breeding programs to carry out marker-assisted selection against the presence of speltoid spike aberrants.

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End-use traits of *Triticale*: Tasks for human utilization

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Triticale is a young crop that is not subject of the EU intervention but its acreage shows a continuously growing trend. Albeit, both of its parentals (sp. *Triticum*, sp. *Secale)* are used for human food, the utilization of triticale as human food is still uncertain. Recent research results show the advantages traits of triticale as a food or food component. Furthermore, decreasing rye production accelerate the industrial and consumer need for high fiber-content, nutritional grains and food products. The main focus of this paper was to study and compare technological and nutritional values of triticale and its parental species. Triticale (cultivars and experimental lines) along with wheat and one rye varieties used in these tests originated from two location (Szeged, Kiszombor) of South Hungary. The samples were studied for important nutritional values and technological parameters (crude protein, crude fat, ash, dietary fibre (DF), arabynoxylans (AX), starch, mineral elements). Most of the grain and flour technological properties (kernel diameter, Test Weight, flour yield, gluten content, Zeleny volume) of the triticale entries positioned in between the parental wheat and rye attributes. Thousand kernel weight (TKW) of triticale cultivar GK Szemes and grain hardness of cv. GK Idus surpassed all other entries. DF contents of triticales were between the rye and wheat controls and found to be strongly affected by location. Total arabinoxylan content (TOTAX) of triticales, on the average, positioned much closer to rye (6%), and in some genotypes significantly expanded it (6.5-7%). In triticale grains, AX (3.3-7.4%) as the main components of DF, was not affected significantly by the location but the genotype-effect was highly significant. It was revealed that beside the fibers, triticale grains were very rich in beneficial elements (Ca, Mg, P, K, Cu, Zn Fe) and the nutrient concentrations showed a wide range of variation. Results suggest that breeders have durable prospect when selecting for high total DF, TOTAX, and for elevated mineral element concentrations. The inappropriate nature of triticale flours *per se* seems to hinder the human utilization. However, for baking industry, blends of wheat and triticale flours may offer appropriate solutions. Blends exploiting favorable attributes of triticale may compose this crop desirable in milling industry and utilization its products on a wider industrial scale. Health conscious consumers trend to use increasingly novel and valuable grain sources and products in their daily food, thus, triticale has a capable prospect in future human diet.

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Session VI

Mapping, Cloning and Beyond

Domestication of barley: Independent mutations in *Btr1* and *Btr2* genes generated a tough rachis that facilitated harvesting of grain

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The grains of wild barley (*Hordeum vulgare* ssp. *spontaneum*) scatter at maturity because the rachis (inflorescence stem) becomes brittle. The transition to inflorescences that retain their grains was the key transition during the domestication of barley and other cereals. However, the domestication processes and molecular mechanisms responsible for this transition in barley are unknown. Here we show that two barley genes for brittle rachis, *Btr1* and *Btr2*, are physically closely linked and encode two independent proteins. Domesticated barley (*H. vulgare* ssp. *vulgare*) arose by independent mutations in the coding sequences of *Btr1* and *Btr2*. Our results demonstrate that brittle rachis represented a new mechanism of separation of grains in barley and its relatives. The identification of the *btr1* and *btr2* loci provides insight into the barley domestication process and a foundation for studies aimed at achieving a mechanistic understanding of how the brittle rachis evolved in cereals.

Effect of temperature on recombination in barley

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An ability to manipulate the process of recombination would potentially enable the improvement of the speed and accuracy of plant breeding and gene cloning. This would be particularly useful in Triticeae crop species where a highly skewed distribution of meiotic crossover events means that up to a third of genes rarely recombine, preventing the generation of novel gene combinations and useful variation that could be exploited in breeding programmes.

One strategy to influence this process is through the use of temperature stress which is well documented to affect recombination frequency. We have carried out a comparative study on the effect of temperature stress on reciprocal backcrosses derived from an F_1 derived from a standard spring barley cross. This work has demonstrated a significant effect of temperature on both recombination frequency and distribution but also that the stress affected male and female meiosis differentially. The heterochiasmy observed can be related to the observed spatiotemporal initiation of recombination in euchromatic DNA in the early stages of meiosis. In addition the differences observed give insights into the malleability of the recombination landscape in barley and mechanisms underlying differences in genetic maps in the Triticeae generally.

A reference sequence of wheat chromosome 3B reveals structural and functional compartmentalization

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We produced a reference sequence of the hexaploid wheat chromosome 3B. We established a strategy that combined several technologies to sequence 8452 Bacterial Artificial Chromosomes pooled by 10 and were able to assemble a pseudomolecule of 774 Mb carrying 7264 protein-coding genes and 85% of transposable elements. Comparative genomics with model grasses revealed that wheat has recently undergone massive inter and intrachromosomal gene duplications. Distribution of both structural and functional features highlighted a striking compartmentalization. Chromosomal extremities, corresponding to regions where meiotic recombination takes place, are enriched in genes originating from recent duplication events, expressed in specific conditions, and with function related to adaptation, which contrasts with the features of the central region of the chromosome. Such reference sequence provides an important resource to support the identification of genes underlying important traits and novel insights into the organization, function and evolution of a complex polyploid genome.

Broad spectrum resistance in wheat: Retrospect and prospects

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The search for and deployment of resistance genes has been an integral part of improvement programs in most crop species for over a century. Introduction of resistance genes from crop gene pools and sexually compatible wild relatives have seen great advances as well as spectacular breakdowns in curbing crop losses caused by a wide range of pathogens. By virtue of the rapidly evolving nature of pathogens due to mutations in virulence gene products or 'effector genes' there is a requirement for improved crop varieties to carry a diverse arsenal of plant defense traits so as to be a step ahead of the co-evolving pathogens. Most resistance genes encode plant immune receptors, and are often short-lived in their usefulness as the pathogen evolves to escape recognition. However, certain forms of plant defense genes provide more durable resistance. In wheat, for example, broad spectrum resistance can be manifested in terms of race non-specificity for a single pathogen species or at a multi-pathogen level. A few genes have been identified that confer adult plant, broad spectrum partial resistance to multiple pathogen species (*Puccinia* sp- rust species, *Blumeria* sp- mildew species and *Bipolaris*/*Helminthosporium* -spot blotch species). More importantly, these multi-pathogen partial resistance genes are components of durable disease resistance with some of these genes continuing to be successfully deployed for nearly 100 years in wheat cultivation.

While very little is understood about how these durable defense genes function, the knowledge being gained from recent cloning of these genes is providing an entry point towards deciphering the functional basis of the broad-spectrum resistance phenotype.

Natural variation at the *Inhibitor of wax 1* (*Iw1*) locus has led to the formation of different haplotypes for glaucousness

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The aerial surfaces of plants are covered by a layer of cuticular waxes, some of which cause a bluish-grey (glaucous) appearance. In wheat, the *Inhibitor of wax 1* (*Iw1*) locus causes a viridescent (non-glaucous) appearance by dominantly inhibiting formation of cuticular waxes. In addition, a previous study had shown that yield and green-canopy duration of a doubled-haploid (DH) population were increased by *Iw1* on average by 4.15% and 1.5 days, respectively, under UK conditions.

We want to identify *Iw1* via a map-based cloning approach: For that we have screened 5,227 plants (10,454 gametes) from two mapping-populations and fine-mapped *Iw1* to a 0.42-cM interval on the short arm of chromosome 2B. We have initiated, but not finished, construction of two physical maps, one in the non-glaucous tetraploid line TTD140 (~870 kb) and one in glaucous line RSL65 (~615 kb).

Despite the incomplete state of the maps it is already evident that the gene complement of both maps is different. We hypothesise that distinct haplotypes have formed at the *Iw1* locus, which would explain the lack of recombination observed in our two mapping populations. Furthermore, based on sequenced contigs of the barley cultivar Morex, the RSL65 haplotype seems to be the evolutionary more conserved haplotype as it shares several genes with the barley contigs, contrary to the TTD140 haplotype. This would also agree with the fact that *Iw1* seems to be a wheat-specific locus that has no orthologues in other cereals. We hypothesise that *Iw1* has emerged after the divergence of barley but before the progenitors of wheat diverged from each other, as *Iw1* has a homoeologue on 2DS called *Iw2*.

We are continuing to construct the 2BS physical maps for TTD140 and RSL65. We are also screening 100 *Triticum turgidum* ssp. *dicoccoides* (TTD) accessions using KASP markers derived from genes of both maps. We hope to identify TTD accessions with similar haplotypes but different phenotypes to create a new mapping population for *Iw1* in order to break the recombination deadlock of the previous two populations.

Construction of physical map for the locus introgressed to bread wheat from *Triticum militinae* conferring powdery mildew resistance

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Introgression from wild relatives is one of the most effective ways to improve wheat gene pool. Recently, a major QTL, QPm-tut-4A, affecting powdery mildew resistance in both seedling and adult plants was mapped to a segment introgressed from Triticum militinae on chromosome 4AL of wheat cv. Tähti. The locus in the mapping population from a cross of Tähti and the resistant introgression line 8.1 was mapped to the distal end of chromosome to a region delimited by markers wmc232 and wmc313 (~10 cM). Unfortunately, in this mapping population, the marker order within this region could not be resolved even using 1200 haplotypes. To further resolve this region a combination of traditional approaches and recent advances in wheat genomics were applied. A 4AL specific radiation hybrid panel and three additional recombination mapping populations were employed to facilitate marker ordering. Survey sequences of 4AL chromosome arm of cv. Chinese Spring and the same arm from the T. militinae introgression were used for efficient maker development. Additionally virtual gene order (GenomeZipper) was established and syntenic genes of rice, Brachypodium and Sorghum were used as markers. The closest flanking markers owm39 and mag2931, which delimit the QPm-tut-4A locus within 0.25 cM, were used for anchoring to the 4AL specific physical map. Two super-contigs 16722 and 16817 with 292 and 338 clones respectively were identified. The contig 16722 was found to carry the QPm-tut-4A locus. The MTP of the 4AL physical map was identified and its 3D pools were sequenced. Using this resource the 4AL physical map contigs were anchored to the GenomeZipper and all 4AL low-copy survey sequences. The MTP anchoring of the target super-contig facilitated saturation of the QPm-tut-4A region with additional 40 markers and delimits the gene region within 0.0625 cM corresponding to two BAC clones. The MTP BAC clones will be sequenced and candidate gene(s) identified. This work has been supported by the grant LO1204 from the National Program of Sustainability I, Internal Grant Agency PrF-2013-003, by the Czech Science Foundation (14-07164S) and by Estonian Ministry of Agriculture.

BARLEYMAP: Unlocking sequence-enriched resources for breeders

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The BARLEYMAP pipeline was designed to map both genomic sequences and transcripts against sequence-enriched genetic/physical frameworks, with plant breeders as the main target users. It reports the most probable genomic locations of queries after merging results from different resources so that diversity obtained from re-sequencing experiments can be exploited. In addition, the application lists surrounding annotated genes and markers, facilitating downstream analyses. Pre-computed marker datasets can also be browsed to facilitate searches and cross-referencing. Performance is evaluated by mapping two sets of long transcripts and by locating the physical and genetic positions of four marker collections widely used for high-throughput genotyping of barley cultivars. Furthermore, genome positions retrieved by BARLEYMAP are compared to positions within a purpose-made genetic map for a RIL population, yielding a gene order accuracy of 96%. These results reveal advantages and drawbacks of current in-silico approaches for barley genomics. A fully-functional barley pipeline is available at http://floresta.eead.csic.es/barleymap. The software can be implemented for any species with similar sequence resources. For this purpose, a customizable, organism-independent standalone version is available for download.

A fast algorithm and software for building ultra-dense genetic maps in the presence of genotyping errors and missing data

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With very large number of markers available for a mapping population, most of the markers will remain inseparable by recombination. In such cases, only one representative per group could be placed on the (skeleton) map; the remaining markers can then be attached to the skeleton. Real situations are also complicated by genotyping errors, which "diversify" a certain part of markers that would be identical in error-free situations. The higher the error rate the more difficult is the problem of building a reliable map. The situation is further complicated by missing data, a usual factor in genotyping-by-sequencing. Our approach to address these problems is based on a simple probabilistic estimation of the proportion of identical markers as a function of the error level when the errors are rare, and of "radius of diversified markers" when the error level is increased. For sample size N and probability p of genotyping errors per marker, the probability that in all individuals both alleles of a marker will be unmistakably identified, is e^{-pN}. Assuming 1% errors within a group of absolutely linked markers, about 1/3 of markers will still remain error-free. For a chromosome of 100 cM, in a population with N=100, the minimum non-zero interval length between markers will be 1 cM, thus the map density cannot exceed 100 markers. With 10,000 markers on the chromosome, only 100 (skeletal) markers can be ordered, whereas the rest will remain linked to the skeletal markers. In our algorithm, we assume that error-free markers can be selected based on the presence of "twins". There is also a probability of an opposite effect, when non-identical markers become "twins" because of genotyping errors. Therefore, a certain threshold is introduced for selection of markers with a sufficient number of absolutely linked copies. The developed algorithm (implemented in MultiPoint software) enables mapping big sets of markers ($\sim 10^5$).

The epigenome of barley: Global profiling of histone modifications in barley chromatin using ChIP-seq

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Epigenetic regulation via post-translational modification of N-terminal histone tails is intrinsically linked to chromatin structure and gene function. Addition of acetyl or methyl groups to amino acid residues in histone tails modulates the interaction between nucleosomes and DNA and can increase or decrease chromatin compaction locally and/or globally in the plant genome. Local chromatin modification can affect gene expression and global modification can be used to partition the genome into euchromatin and heterochromatin. Chromatin immunoprecipitation followed by Next Generation Sequencing (ChIP-seq) is a method that allows the genome wide association of DNA with modified histones to be assayed. Here, we present a ChIP-seq protocol for barley to reveal the sequences that are associated with thirteen different histone epitopes in chromatin extracted from whole barley seedlings. We have also validated three peak calling software packages and will present an optimised protocol for deducing genome-wide occupancy data for modified histones. Lastly, we will present our preliminary results on the relationships between histone modification and gene expression, chromatin compartment and gene evolutionary rate in barley.

Genome-wide association mapping reveals novel insights in the genetic architecture of agronomic and quality traits in rye hybrids

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Among the small grain cereals, rye (*Secale cereale* L.) is unique in terms of his outbreeding lifestyle and elevated tolerance to frost, drought, and marginal soil fertility. This multi-purpose crop is an invaluable part of crop rotation systems and contributes mainly in European agroecosystems to increase crop species diversity. In Germany, hybrid breeding resulted in a substantial gain of grain yield and other traits and keep's rye competitive in modern agricultural production systems. Understanding the genetic basis of various physiological, developmental and morphological traits provides novel options to improve the yield, quality and sustainability of rye. Here we report on the results of a genome-wide association study based on a genotyping-by-sequencing approach and intensive phenotyping in a breeding population of hybrid rye. We have used DArTseq as a complexity reduction method to efficiently target 15,386 SNPs and almost 40,000 presence/absence variants (PAVs) in elite rye germplasm and integrated more than 16,000 markers covering 1,946 cM of the rye genome in the recently established transcript map of rye. We have estimated the average linkage disequilibrium to decay below the critical level within a map distance of 3-4 cM. A set of 197 and 320 experimental hybrids have been phenotyped in 2010 and 2011, respectively, for 8 agronomical and quality traits including grain yield. A novel, cross-validated NIR calibration allowed us to predict the content of crude protein, starch, water-extractable arabinoxylan and total arabinoxylan content in rye grains with unprecedented accuracy. The experimental hybrids revealed significant phenotypic variation for each of the assessed traits. All phenotypic data did not significantly deviate from normal distribution. The intensive phenotyping led to high heritability estimates for all traits including water-extractable arabinoxylan content. We have integrated significantly ($P < 0.05$) associated markers in the recently published genome zipper of rye and identified genes, which are known to genetically control individual QTL. The identified marker/trait associations provide a first step towards a targeted molecular characterization and utilization of genetic resources for precision breeding of hybrid rye varieties.

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Mapping-by-sequencing accelerates forward genetics in barley

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Mapping-by-sequencing has emerged as a rapid and powerful technique for genetic mapping of Mendelian and quantitative traits in several plant and animal species. As this resequencing-based method requires a reference genome to infer the order of genetic markers, its application to large and highly repetitive plant genomes, for which only fragmented genomic sequence information is available, remains challenging.We have performed exome sequencing of phenotypic bulks of a mapping population of barley segregating for a mutant phenotype that increases the rate of leaf initiation. Sequence reads were mapped against a partial gene space assembly of one parent of the population and detected variants were put into a genomic context by the integrated physical and genetic map of barley. The comparison of read depth in mutant and wildtype pools identified a candidate gene, which was confirmed by TILLING and resequencing several independent mutant alleles. This gene is a member of the cytochrome P450 family CYP78A and homologous to rice PLASTOCHRON1.We have demonstrated how the current genomic resources of barley in conjunction with reduced representation resequencing can supply the necessary infrastructure for mapping-by-sequencing. Our approach may be adopted by other map-based cloning projects in barley and other species with large and complex genomes.

Analysis of highly repetitive BAC clones reveals rapid evolution of super-variable gene clusters in Triticeae

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Sequencing of minimum tiling paths (MTPs) of BAC clones is the most robust strategy to obtain the complete sequence of a genome. However, MTP sequencing and sequence assemblies reach their limits in large segmental duplications where the duplicated units are longer that the BAC clones. In the physical maps of barley and wheat we have identified numerous regions which must contain arrays of very large repeat units (>100 kb), because (i) LTC and FPC assemblies indicate an extremely high coverage with BAC clones, and (ii) sequence comparison of BACs from within these "blobs" indicate the presence of extremely large duplicated segments. These repeat arrays must be of relatively recent origin because individual repeat units are extremely similar (less than one SNP per kb) to each other. One such region on chromosome 3H contains a cluster of receptor-like kinase (RLK) genes. We found that pairs of RLK genes plus dozens of kb of flanking repeat sequences have been recently amplified to form an enormous repeat array consisting of at least 8 units, each one with a size of over 100 kb. Individual repeat units differ from each other by only a few SNP per kb. This high sequence similarity allows a rapid growing or shrinking of the cluster through unequal crossing-over, making it a potential hotspot for copy number variations. Interestingly, the orthologous region in rice also contains large clusters of RLK genes and is one of the most dynamically evolving regions in the genome. We are currently analyzing whether similar molecular mechanisms drive the dynamics of these clusters in both species.

Session VII

Future Challenges and Innovations

Innovations and challenges in crop genomics

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Conventional crop breeding essentially rests on repeated cycles of crossing and selection. This approach has warranted the development of superior cultivars over the past decades. However, it is only sustainable, if the genetic diversity that is lost in the process of selection is adequately replenished by introducing novel diversity into the genepool. *Ex-situ* conservation of plant genetic resources represents the major backbone to maintain the intraspecific diversity of many important crop plant species. At present about 7 million seed samples are stored in far more than 1000 *ex-situ* collections worldwide. Undeniably, the vast diversity resting on the shelves of genebanks has been tapped into only marginally.

Hence, genebanks are increasingly expected to provide informed access to their genetic resources. At the highest level of resolution, this means that each genebank accession is tagged with information on individual alleles along with their phenotypic effects. The majority of crop genomes are characterized by their large size and inherent complexity impinging upon molecular analysis, meiotic recombination and, sometimes, their amenability to genetic modification. However, these limitations have been increasingly overcome by technical advances in several key areas. Progress will be reviewed by illustrating examples of the IPK research program on barley and wheat regarding (i) structural and functional genomics, (ii) phenotypic cataloging of accessions using automated imaging and (iii) novel biotechnological approaches that will provide entry points for crop improvement. While the application of novel technology opens up a wealth of entry points for genetic analyses, it also generates and amasses humongous streams of data. Therefore, integrated concepts of data management and analysis will need serious consideration when aiming at the exploitation of novel technologies for the systematic phenotypic and genotypic characterization of genebank collections.

Integration of DArTseq genome profiling and KDDart IT platform into breeding of Triticeae crops

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Diversity Arrays Technology (DArT™) was developed over a decade ago to enable crop breeding with utilisation of the whole genome profile information. In the last three years we have launched a new service using DArT complexity reduction methods combined with Next Generation Sequencing platforms. This new (DArTseq™) platform has been applied to over 100,000 of wheat samples and all other crops from Triticeae tribe. We have established a large database of sequence variants in DArTdb, LIMS/database system design to manage molecular marker production and storage. We have also developed a range of algorithms for genetic map and consensus map construction, QTL and GWAS analysis and successfully benchmarked a number of new algorithms for phenotype prediction against those most commonly used in the field of Genomic Selection. A few examples of such applications will be presented. These algorithms are deployed in our service, offering our clients “one stop shop” for marker data production and analysis.

We will present a model of application of scalable, inexpensive whole genome profiling combined with efficient Information technology in modern breeding. This approach is based on our KDDart system for data storage and analysis, which integrates molecular information with field data and environmental characteristics. A few User Interfaces for the platform will be presented with the main emphasis on KDMan data management component and KDCompute data analysis application. The challenges and the opportunities for organisations transiting from traditional crop improvement to highly digitized breeding will be discussed.

Genomic selection in wheat: Accuracy in cross-population prediction

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With the expected development of thousands of molecular markers in most crops, the marker-assisted selection theory has recently shifted from the use of a few markers targeted in QTL regions (or derived from candidate genes) to the use of many more markers covering the whole genome. Provided that a sufficient level of linkage disequilibrium exists between the markers used for genotyping and the true genes underlying QTLs for complex traits, these genome wide markers can be used to predict the true breeding value (Meuwissen et al. 2001). This can allow shortening selection cycles (particularly when accurate phenotyping requires time as in perennial species), therefore increase genetic gain per unit of time (Heffner et al. 2009). To be useful for breeding purposes, the accuracy of this Genome Estimate of Breeding Value (GEBV) should not be worse than the estimation based on phenotype, which is not always the best predictor of Breeding Value, particularly in the presence of GxE interactions. Moreover, breeders may wish to have robust predictions, i.e. which are stable from one germplasm to another, in order to establish a common reference population to train prediction equations, then apply them on a range of genetic materials. This investigation uses three different sets of advanced breeding lines from two breeding programmes (one public, one private) to assess prediction accuracy (Iwata and Jannink 2011) by either 1) cross validation within a single training population, 2) cross validation by resampling within a mixture of 2 or 3 breeding populations and 3) cross validation using resampling within one population for training and resampling within another populations for validation. Results show that prediction accuracy is much lower when using one population as training set and another as target set, as compared to within population cross validation. This may be explained by a lower level of genetic relationship among than within population. Selecting a subset of most of training lines most related to the target set leads to improved accuracy.

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Genomic Selection: Training populations and GxE

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Two complementary approaches to molecular breeding are marker assisted selection (MAS) and genomic selection (GS). MAS can be used to transfer major genes and both methods can be used in marker-assisted recurrent selection (MARS) schemes. GS is the simultaneous use of genome-wide markers to increase accuracy of performance prediction for both phenotyped and unphenotyped individuals. In GS, a training population related to the breeding germplasm is genotyped with genome-wide markers and phenotyped in the target population of environments. That data is used in a prediction model to estimate the breeding values of unphenotyped candidates. Consequently, design of the training population is critical to the accuracy of the prediction models. Because of reduced selection cycle time, annual genetic gain for GS is predicted to be two to three fold greater than for a conventional phenotypic selection program, even with only modest prediction accuracy. Recent studies have illustrated the importance of close relationship between training and breeding populations. There is a tradeoff between population size and relationship that affects prediction accuracy. Prediction models can incorporate performance over multiple environments and assess GxE effects to identify a highly predictive subset of environments. We have developed a methodology for unbalanced datasets using genome-wide marker effects to group environments and identify outlier environments. In addition, environmental covariates can be generated using a crop model and further used in a GS model to predict GxE in unobserved environments. In that model, marker effects are fitted as a function of environmental covariates. This model increases prediction accuracy and facilitates performance prediction in climate change scenarios. Current research is focused on optimizing the training population to improve efficiency and increase prediction accuracy in terms of genotypes, experimental design, and environment sampling.

The IWGSC: Building the sequence-based foundation for accelerated wheat breeding

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The International Wheat Genome Sequencing Consortium (IWGSC) was established in 2005 with the goal of securing a high quality, reference sequence of the bread wheat genome. The large size and complexity of the hexaploid bread wheat genome have posed severe challenges for the development of genomic resources aimed at accelerating wheat breeding improvements. To reduce the complexity of sequence assembly and chromosome assignment for the allohexaploid, highly repetitive, 17Gb bread wheat genome, the IWGSC is developing physical maps, survey sequences, and pseudomolecules for individual chromosomes flow-sorted from aneuploid cell lines of the reference variety, Chinese Spring 42. The consortium's milestone-based strategy has provided breeders and plant scientists with access to an increasing array of tools and resources without having to wait for the completed sequence. Physical maps and Minimum Tiling Paths (MTPs) have been completed or are underway for all chromosomes. Most recently, the IWGSC completed a first draft survey sequence of the genome based on Illumina short read sequence data which was annotated to assign gene models and public marker sets to individual chromosomes/chromosome arms. High quality, BAC-based sequencing of chromosome 3B and its analysis was completed in 2013 by INRA/ Genoscope. BAC-based sequencing of other chromosomes is ongoing in several other countries and efforts to secure funding for the remaining chromosomes are underway. Information about the various consortium projects can be found at www.wheatgenome.org and current wheat genome resources can be accessed from the IWGSC repository at http://wheat-urgi.versailles.inra.fr/Seq-Repository. An overview of the strategies and results from IWGSC projects will be presented.

Large scale SNP genotyping with optimized marker sets for cost-efficient wheat breeding using molecular markers

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Through the development of the 90K Illumina Infinium genotyping array and other Infinium genotyping arrays, it has now become routine to generate a wealth of genotyping data for individual wheat lines. However, many of the genotype data generated in this way create redundant information in wheat breeding material and varieties. With genotyping data generated from a large set of lines derived from various sources and countries and mapping information for more than 50000 markers, we have investigated in detail the extent of LD and haplotypes in elite wheat material in order to identify haplotype-specific markers of high quality. Furthermore, we have integrated data from known marker/trait associations. Taking all this information together, we have subsequently generated an optimized genotyping array for routine use in wheat genetic analyses and breeding including genomic selection. This array can be used at much reduced costs compared to other arrays and without much loss of information. In parallel, we have generated an optimized marker set based on individual SNP markers (KASP) for variety identification, variety purity analysis, marker-assisted backcrossing and other purposes.

Efficient and heritable genome engineering in barley using customized TALE-nucleases

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Site-specific genome engineering is a breakthrough technology that facilitates the functional validation of genes and offers versatile novel possibilities of crop improvement. Aiming to establish genome engineering as a viable tool for cereals, we generated and expressed *GFP*-specific transcription activator-like effector nucleases (TALENs) in barley lines harbouring a single copy of *GFP* that was used as experimental target sequence. Mutant plants were produced *via* Agrobacterium-mediated transfer of a functional pair of TALEN constructs to pollen undergoing embryogenic development. When mutagenesis events occurred prior to spontaneous or artificially triggered duplication of the initially haploid pollen-derived tissue, non-chimeric and instantly homozygous mutants were obtained, as was indicated by non-segregating progeny. In this approach, over 20% of TALEN-transgenics proved to be target gene knock-out lines. In an alternative setup, the two TALEN units required for site-directed cleavage activity were separately used to produce homozygous transgenics each carrying only one unit of the TALEN pair in *GFP* background. Crossings of these plants resulted in pair-wise combination of both TALEN units, which entailed their activation during early zygotic embryogenesis in the hybrid caryopses. While sequencing of target-specific PCR amplicons revealed multiple mutant alleles in each of the analysed seedlings, the *GFP* wild-type allele was only rarely detectable, suggesting an unprecedented efficiency of site-directed mutagenesis in plants. The comparative analysis of a large number of independent mutations revealed an indel size of between 1 and 15 nucleotides in two thirds of the alleles obtained. We thus anticipate that the method may not only be utilized for the generation of knock-out mutants but also of plants carrying alleles with attenuated or modified functionality. In addition, these results may pave the way for the establishment of even more sophisticated procedures aiming to precisely edit cereal genomes based upon double strand break repair *via* homologous recombination.

Sustainable agricultural production in Cyprus and the crucial role of density-independent varieties under global climate change

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During the past three decades, on average 13% of the rain falling on the Republic of Cyprus has been converted to agricultural products, while some 70% of the rain water that flows to dams has been used for irrigation. Climate change projections indicate that Cyprus, similarly to other water-stressed countries, is becoming hotter and drier. This has a significant impact on the future of agriculture. The aim of the AGWATER project consortium is to provide recommendations for climate change adaptation for the agricultural sector in Cyprus and the wider Mediterranean region. The project has developed an agro-meteorological database for the period 1980-2010 and has mapped Cyprus' agro-climatic zones. Future climate data have been statistically downscaled using data from 3 different Regional Climate Models. High resolution gridded rainfall and temperature data have been developed for 1980-2010 and for 2020-2050 (Camera et al, 2013). Three global energy price scenarios have been prepared to assess future water prices and production costs. A new 1:50,000 soil map is generated with the use of an intelligent classification algorithm (Random Forests), and a large geo-referenced database prepared. The effect of seasonal precipitation and temperature extremes were analyzed for 30 years of barley trial data, with five varieties at three locations. The novel concept of density-independent varieties (Fasoula and Fasoula, 2000; Fasoula, 2012) is shown to have a major role to play in securing cultivar adaptation under global change.

Acknowledgements

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Structure and evolution of the microbiota thriving at the barley root-soil interface

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Roots of land plants nourish rich and diverse microbial communities in the vicinity of and within their tissues. These communities, designated rhizosphere and root microbiota, establish associations with their host that appears symptomless at first glance, possibly representing a continuum of symbiosis ranging from commensalism to mutualism. Consistently, members of the microbiota contribute, under laboratory conditions, to indirect pathogen protection and enhanced mineral acquisition for plant growth. However the molecular mechanisms regulating plant-microbiota interactions remain still largely unknown.

We have profiled the rhizosphere and the root bacterial communities retrieved from soil-grown, wild, landrace and modern accession of barley using a 16S rRNA gene amplicon pyrosequencing approach. Phylogenetic assignment of the generated sequencing reads revealed that the microbial communities thriving at the barley root-soil interface appears to be gated, as indicated by a narrow phylogenetic composition, largely dominated by members of the families Flavobacteriaceae, Rhizobiaceae and Comamonadaceae Interestingly, constrained ordinations and linear model analysis revealed that host microhabitats (i.e. root and rhizosphere) are the major determinants of the barley microbiota, while the host genotype determines its ribotype profiles to a limited extent, suggesting that the structure of the barley microbiota is a conserved trait in the tested accessions.

To gain insights on the molecular cues underlying microbial recruitment at the root-soil interface, we reconstructed 20Gbp of the barley rhizosphere metagenome. Interestingly, we observed that ~10% of the protein families encoded by the barley microbiome display a ratio between non-synonymous and synonymous substitutions (ω) significantly higher than the mean of the sequenced metagenome. Intriguingly, these data might indicate an evolutionary pressure on proteins required for the microbial colonisation of the rhizosphere. Remarkably, proteins previously reported in host-microbe interactions studies (e.g. putative bacterial effectors) showed a ω significantly different across genotypes, suggesting that host genetic traits contribute, at least in part, to bacterial functional diversification in the rhizosphere.

Our work provides a foundation for future studies aiming at the characterisation of the genetic bases of plant-microbiota interactions and, in the long term, the introgression of the microbiota metabolic potential into plant breeding.

Comparison of different spatial models for field trials in stage-wise analysis for genomic selection in rye (*Secale cereale* L.)

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Genomic selection is becoming a daily tool for plant breeders. It makes use of genotypic data for selection decisions. We used spatial models in the phenotypic analysis stage to obtain better estimates, and thus improved prediction abilities for genomic selection. We used a real dataset of a multi-environment trial in rye, which was connected between years only through one check. In total, 1610 S_2 inbred lines evaluated as testcrosses were phenotyped and genotyped with 11000 single nucleotide polymorphism markers.

The aims of this study were to explore genomic selection as a tool for model selection, to analyse an experiment weakly connected across years using different stage-wise approaches, and to assess the advantage of using spatial models for the predictive abilities of genomic selection.

Predictive abilities can be used to select spatial models. They did not follow the same trend as the traditionally used Akaike information criterion, but favoured in the end the same models.

Using complex spatial models did not improve significantly the predictive ability of genomic selection, but using row and column effects yielded the highest predictive abilities of all models.

In the case of multi-environment trials poorly connected between years, analysing each year separately and fitting year as a fixed effect in the genomic selection stage yielded the most realistic predictive abilities.

Making predictions using weakly linked datasets is of utmost interest for plant breeders. We provide an example with suggestions on how to handle such cases. Rather than relying on checks we show how to use year means across all entries for integrating data across years.

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Honorary Session

Improvement of hybrid rye for yield traits and drought tolerance by molecular breeding

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Winter rye (*Secale cereale* L.) is an important cereal, particularly in Germany, Poland, Belarus, and the Russian Federation grown on about 4 million hectares in 2012. Rye is used for bread making, as feed, and in Germany also for bioenergy production, i.e. biomethane and bioethanol. Changing demands of the market, globalization of breeding, and climate change afford new breeding purposes or a different emphasis on already existing goals, like drought tolerance, grain and biomass yield, quality traits. Hybrid technology combined with marker techniques should help to fasten selection and improve selection gain.

We analyzed two introgression libraries comprising in total 78 lines and several segregating mapping populations with >200 progenies with multi-environmental phenotyping and DArT, SSR, and SNP genotyping for up to eight traits, including grain and biomass yield, plant height, thousand-kernel weight, protein, starch, and pentosan contents under rainfed and irrigated conditions. For all traits, significant genotypic variation with moderate to high heritabilities was found in both elite mapping populations and introgression libraries. Variation for pentosan content was restricted due to an intensive selection for baking quality. Genetic correlations of grain yield between drought and rainfed conditions were narrow (r=0.9), but low between grain and biomass yield (r=0.3) under optimal conditions. Some cross-validated QTL, especially for thousand-kernel weight and quality traits, but also for grain yield, had major effects and were highly environmentally stable. For drought tolerance, specific QTL were already available in elite populations, but should be supplemented by additional loci. Genetic architecture of these traits and the efficiency of both population structures for detecting QTL will be compared. The results provide good prospects for future genome-wide prediction analyses in hybrid rye breeding.

Genomic selection in hybrid rye breeding

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The key feature of genomic selection (GS) is the estimation of genomic breeding values (GBVs) from genotypic and phenotypic data in a training population and the subsequent prediction of breeding values of untested individuals with maximum accuracy. While substantial research has been dedicated to the incorporation of GS into hybrid maize breeding, the methodology still needs to be specifically tailored to applied rye breeding programs.

The objectives of our study were to i) compare the prediction performance of pedigree-based (PBLUP) and genomic best linear unbiased prediction (GBLUP) within and across two interconnected rye breeding cycles, and ii) make inferences on the potential of GS for improving economically important traits in hybrid rye breeding using cross-validation. The study comprised data of 900 and 820 S2 inbred lines evaluated as testcrosses in replicated field trials at multiple locations in Germany and Poland over two years. Testcrosses were phenotyped for the traits grain dry matter yield, plant height, and thousand kernel weight, and genotyped with 10,416 single nucleotide polymorphism markers. Pedigree information was available on 10 generations.

With respect to prediction performance, GBLUP clearly outperformed PBLUP within selection cycles and yielded predictive abilities at intermediate to high (0.54-0.71) levels. In across cycle prediction we observed low to intermediate predictive abilities (0.28-0.48). Here, the relative advantage of GBLUP over PBLUP was slightly less pronounced. In the prediction across breeding cycles, GBLUP yielded an average predictive ability of 0.39 across all traits.

We conclude, that the superiority of GBLUP over PBLUP and the achieved prediction performances promise a successful implementation of GS into hybrid rye breeding programs. In order to further elucidate components of prediction performance and to increase across cycle predictive abilities we will extend our analysis to models accounting for the population substructure of the S2 selection candidates. Further we will investigate the influence of the hybrid tester on prediction performance.

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Poster Presentations

Session I

Genetic Resources

Molecular characterization of vernalization response genes in wheat genotypes

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This work was the first attempt to characterize the vernalization response (*Vrn*) genes in thirty wheat (*Triticum aestivum* L.) genotypes conserved in the National Gene Bank, Agricultural Research Center, Tripoli, Libya.

PCR was carried out to amplify DNA fragments associated with Vrn genes using four specific primers to determine the presence or absence of dominant and recessive alleles in the three main Vrn loci (*Vrn-A1, Vrn-B1, Vrn-D1*). Standard DNA (DNA ladder) was used to determine the fragments lengths generated by the different primers in the PCR analysis. The dominant allele Vrn-A1a was present in only 6 genotypes from the 30 genotypes studied. The rest of the genotypes amplified one fragment of approximately 500 bp indicating that these genotypes either carry the dominant allele (*Vrn-A1b*) or the recessive allele (*vrn-A1*), but we could not differentiate between the two alleles in this work. The second primer associated with the *Vrn-A1c* allele could not amplify any DNA fragments, indicating the absence of the target allele in all the genotypes studied.

The results from the third primer associated with the gene *Vrn-B1* showed that 25 out of the 30 genotypes carry the dominant allele *Vrn-B1*. The fourth primer linked to the gene *Vrn–D1* amplified fragment with approximately 1671 bp in only five genotypes.

Wild wheat relatives and CVS on the Fe, Zn CD content evaluation in breeding (tolerance)

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For Kazakhstan, the most important production of ecologically pure grain products, as grain is the main agricultural product of the country. The most ubiquitous pollutants in Kazakhstan are copper and cadmium, along with lead and zinc. There are different opinions on the effect on the adaptive plant metabolism.

Laboratory researches on influence of ions of Zn2+, Cu2+, Cd2+of metals (20 mg/1 in a nutrient medium) on changes of physiological & biometric parameters growing of 7 day sprouts of wheat were carried out in 2010-2013. Comparison of metal resistance of plants carried out on the basis of Wilkins coefficient calculation.

It is established that ions of Cu2+ and Cd2+ render stronger inhibiting effect for growing of roots and vegetative mass, free proline content of all studied versions of wheat. The Wilkins coefficient was higher for facultative wheat in winter crops in comparison with spring at cultivars "Ruta" and "Kazahstanskaya 10".

Under the influence of HM there was a considerable accumulation of free proline for varieties Intensivnaja and Pamjat' 47 in winter form, especially under the influence of Cu2+ and Cd2+. The made experiments show the role of proline as endogenous regulator stress stability in reaction to TM action. Researches on use of free proline as a sign for pre-breeding and definition of genotypes - donors of metal stability of wheat are expedient.

One of such samples is the wild forms of wheat. Cd content in the grain in reproduction for wild relatives (16 *Triticum* spices) and *Aegilops* varies from less 20 to 25 mg/kg (*T. persicum* and *T. kiharae*) and in the second reproduction from less 20 mg/kg to 24-26mg/kg (*T. spelta, T. polonicum, T. militinae*) and maximum to 35mg/kg among *Triticum* for *T. dicoccum.* Among the 5 samples of *Aegilops* origin only *Ae. cylindrica, Ae.squarossa* was differing with a high Cd content in the grain (30 and 54 mg/kg).

High-density genetic map of durum wheat × wild emmer wheat based on the 90K iSelect SNP genotyping assay

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A high density genetic-map of tetraploid wheat was constructed based on 140 F_6 recombinant inbred lines (RILs) derived from a cross between elite durum wheat [*Triticum turgidum* ssp. *durum* (Desf.) MacKey] cultivar 'Svevo' and wild emmer wheat [*T. turgidum* ssp. *dicoccoides* (Körn.) Thell.] accession 'Zavitan'. The wheat 90K iSelect genotyping assay was used for genotyping of the RILs, resulting in 10,306 polymorphic markers. In addition, we added 26 microsatellite markers as anchors. About 10% of the polymorphic SNP markers showed a small but significantly higher heterozygosity level than expected ($P \leq 0.05$). In contrast, the 26 SSR markers showed the expected ~3% heterozygosity. These results suggest that the SNP assay may have a small technical bias. To construct a stabilized genetic map, 2831 markers interfering with map stability were removed by the MultiPoint-ultradense package. The remaining 7,475 markers (1,046,500 data points) were grouped into 1,199 loci (skeleton markers) in 14 linkage groups. Map length was 2,026 cM with an average distance of 1.7 cM between adjacent skeleton markers and individual chromosome-length ranged between 106 cM for chromosome 4B and 195 cM for chromosome 5A. Interestingly, the SNP markers showed equal distribution between the B-genome (51.5%) and the A-genome (48.5%). The recently sequenced barley genome was used to study the synteny between the two species. This comparison revealed 4,168 syntenic markers (>85 % identity and >75% length match) located on orthologous barley and wheat chromosomes. The rest of the markers (445) that matched the barley genome were non-syntenic including loci in known translocation regions in wheat chromosomes 4A, 5A and 7B. This SNP-based genetic map with substantial association to the barley genome sequence provides a useful groundwork for genetic analyses of important quantitative traits, positional cloning, and marker-assisted selection, as well as for comparative genomics and genome organization studies in wheat and other cereals.

Genetic control of heading and flowering time in wheat

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Understanding of genetic control of heading and flowering time is very important for adaptation of new varieties. Determination of novel QTLs and alleles would have a great impact on breeding strategies to meet future climate changes. Association mapping is the most perspective method for exploration of the entire genome. Microsatellite markers were used to evaluate genetic structure in order to investigate marker-trait associations (MTA). Population consisting of 283 wheat varieties was used for genotyping and phenotyping. It is the part of Small Grain Department core collection of Institute of Field and Vegetable Crops in Novi Sad, Serbia. Heading and flowering time were recorded during five growing seasons. Genomic DNA was extracted with modified CTAB method. The PCR reactions were performed using fluorescent labeled primers and PCR products were sized by capillary electrophoresis using Genetic Analyzer ABI 3130. Genetic diversity was calculated by Microsatellite Tool Kit and association study was performed using general linear model (GLM) and mixed linear model (MLM) in software Tassel 2.1. The Q matrix was performed according to the results obtained in program Structure v.2.3.4 based on six subpopulations. The average PIC value was 0.68. Significance level of 0.01 was used to identify marker-trait associations. The total number of marker-trait associations for heading time using GLM method was 36, whereas for flowering time it was 35. The MLM method has shown better resolution than GLM approach with 12 detected MTAs for heading and 15 MTAs for flowering time. For both traits investigated, six markers (WMC167, WMC44, GWM294, CFA2086, WMC28, and WMC407) were significantly associated in more than three growing seasons. The significant associations of both traits were located on the same chromosome groups (2A, 2D, 1B, and 5B).

The main goal of this paper was to determine genetic diversity in the chosen material and to find stable marker-trait associations involved in the expression of two important adaptive parameters: heading and flowering time.

A strategy to identify regions in multi-environment trials: The case study of wheat in North-Western Europe

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Genotype-by-environment interaction (GxE) is commonly observed in wheat trials and often GxE involves crossovers, i.e. changes in the ranking of the genotypes between testing sites. The changes in the ranking of genotypes complicate the selection of new varieties. To select adapted genotypes, breeders perform multi-environment trials (MET), using testing sites that represent target production environments. Response to selection can be improved by subdividing the testing sites in regions (mega-environments), where ranking of genotypes within regions exhibits more consistency than between regions. The grouping of testing sites into regions is a relevant task, for what various approaches based on fixed linear-bilinear models have been proposed (SHMM, AMMI, GGE). Mixed model approaches for the identification of regions also are potentially powerful alternatives, although in general they require a higher ingenuity in statistical modelling than standard fixed models do. We compare here two strategies for grouping testing sites in regions, one based on a full mixed model analysis, and one based on a relatively simple, yet robust two-step approach based on an AMMI model. Within years, AMMI models are fitted to genotype by location tables of means, and the AMMI predictions are used to cluster locations. Locations that consistently cluster together across years are assigned to the same region. The mixed model approach utilizes the parameters of a factor analytic model to cluster locations in regions. Correlated response to selection is used to assess the advantage or not of classifying locations in regions. We illustrate the approach with yield data from official variety trials in the period 1995-2012, in Denmark, Germany, the Netherlands and the United Kingdom. We identified regions in Denmark, Germany and the United Kingdom, and these regions coincided with latitudinal and longitudinal gradients, with the regionalization being especially important in Denmark.

The EU-FP7 WHEALBI Project: Wheat and barley legacy for breeding improvement

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WHEALBI is granted 5 M€ by EU-FP7 for 5 years starting January, 2014. It involves 18 partners (8 academics, 7 industry /SME) in 9 countries and aims at improving European wheat and barley production in competitive and sustainable cropping systems. Germplasm will be selected and characterised by next-generation-sequencing. Adaptive traits will be evaluated in both transnational field experiments and precision phenotyping platforms. Germplasm will be stored in a bio-repository and associated data in knowledge bases that will represent a valuable legacy to the community. Whole genome association scans will be conducted for several traits, signatures of adaptive selection will be explored, and allele mining of candidate genes will reveal new variation associated with specific phenotypes. Pre-breeding tools will be developed to optimize the efficiency of allele transfer from unadapted germplasm into elite breeding lines. New methodologies will explore how to optimally exploit the large amount of new genotypic and phenotypic data available. Ideotypes with improved yield stability and tolerance to biotic and climatic stresses will be designed and and provide proof of concept of the efficiency of genome and phenome assisted selection. Ideotypes and reference varieties will be evaluated in innovative cropping systems, particularly organic farming and no-till agriculture, and an economic evaluation will be conducted. Results will be disseminated to a broad user community, highlighting the benefits and issues associated with the adoption of sustainable wheat and barley crop production. WHEALBI aims to help the EU remain a major actor in world small grain cereal production while addressing the pressing global priorities of improving food quality and reducing environmental impact.

Genetic diversity and population structure of wheat genotypes for rust resistance by SSR

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Wheat (*Triticum aestivum* L.) is among the most important cereal crops and major staple food of many peoples around the globe. It ensures food security for the increasing population of world. Wheat is vulnerable to many diseases. Among them, rusts are the major yield limiting factors to reduce overall wheat production. The main rusts are of three types which include stem rust (Sr), yellow rust (Yr), and leaf rust (Lr) caused by different fungal strains. SSR markers were used for genetic diversity analysis of wheat rust resistance genes. Available wheat germplasm was screened against Sr, Lr and Yr resistance genes. Seven polymorphic SSRs of 11 were used dete cted alleles, ranging from three to four alleles per locus. All the accessions were categorized into four groups A, B, C and D on the basis of their morphological performance. The average number of alleles per locus was 3.545, 2.818, 3.818 and 2.636 for group A, B, C and D. Gene diversity and heterozygosity are high at locus Sr-25 but its major allele frequency is low while PIC is high at loci Lr-34 and Yr-18. Study also confirmed various linked genes for rust resistance in wheat. Gene diversity, heterozygosity and PIC are lowest at locus Sr-39 but its major allele frequency is highest. Gene diversity increased with increase in the number of alleles that had a low major allele frequency at a locus. Seven polymorphic loci effectively discriminated 360 taxa (accessions) into six major clusters via principal component and population structure analyses. Significant deviation of F_{ST} from zero in six suggested populations for seven loci indicated population differentiation and limited gene flow among them. A reduced median network established that taxon MaxiPak-65 is primitive while Chanab-70, SA-42 and Yecora-70 were also found the progenitors and sharing rust genes to Pakistani wheat. AARI-11 showed high gene diversity and PIC compared to the rest of clades thus have broader genetic base and can be utilized in breeding program. These SSRs are a valuable resource and may be useful in marker assisted wheat breeding.

Black barley as a means of mitigating deoxynivalenol contamination

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Fusarium head blight (FHB) is a destructive disease of barley *(Hordeum vulgare* L.) in many countries. The disease, in Canada, is caused principally by *Fusarium graminearum* Schwabe, and can result in mycotoxin, deoxynivalenol (DON), contamination in the grain. The objective of this study was to determine if black barley is more resistant to DON accumulation than yellow barley as the former contains a high level of phenolic compounds. In one experiment, 100 recombinant inbred lines (RILs) were derived from each of the two barley crosses: AC Klinck/CH9403-2 and AC Legend/CH9403-2 with half of them being black and the other half yellow. These lines along with their parents were evaluated for resistance to DON accumulation under natural infection conditions at Harrington (Prince Edward Island) for three years (2005-2007). They were also evaluated for resistance to FHB incidence and DON accumulation under artificial inoculation conditions at Ottawa (Ontario) in 2005 and 2006 and at Hangzhou (China) in 2005-2006. The results of this experiment showed that black RILs on average contained 17-59% less DON than yellow RILs under natural conditions in both crosses in two of the three years and that black RILs had 2-20% lower FHB incidence than yellow RILs in both crosses in two of the three tests and contained 16-18% less DON in one test. In another experiment, 26 hulless accessions, one FHB-resistant check (Chevron) and one FHB-susceptible check (Stander) were planted in a randomized complete block design with four replications at Ottawa in 2011 and 2012. The results showed that black accessions on average contained 46% less DON than yellow accessions over the two years. In conclusion, black barley can be used as a means of mitigating DON contamination.

Genetic integrity of rice seeds during ageing and gene bank storage by SSR analysis

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Genetic alterations during ageing of rice seeds were analyzed using SSR markers. In the first experiment, seeds of 10 accessions of rice were subjected to artificial ageing in chambers conditioned to 55°C and 72±2% RH for 72 hours. In the second experiment, seeds of 4 accessions of rice stored in the NACGRAB gene bank, Nigeria in 2011 at 5± 4°C were compared with seeds of the same accessions freshly harvested in 2013. The objectives of this project were to evaluate ageing induced genetic changes and to establish physiological thresholds for loss of genetic integrity for rice seeds. Data were collected on seed germination and a seed vigour index was estimated. Genetic changes during the ageing course were evaluated by SSR analysis using a Direct™ PCR kit. Genetic drifts and distances were estimated using PAST™ software and percentage genetic integrity was estimated from the genetic distance matrices. At 72 hours of ageing, seed germination percentage declined to 54.2% and vigour index 1.38 coinciding with the lowest estimate of genetic integrity (99.5%). Probability of genetic drift during ageing as revealed by primer RG178 was significant ($P<0.005$) for CG-14, IR-64 NERICA-1 and NERICA-L-34 and WITA-4. Differences in genetic distances were highest and estimate of genetic integrity was least in NERICA-1. Germination of seeds in gene bank after one and two years storage was above 99% and there were no significant genetic drifts for all accessions. The implications of result for gene bank activities will be discussed.

B1K Fanomics: Investigation of canalization against environmental stress in *Hordeum spontaneum*

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Canalization, the maintenance of a character in the presence of environmental change is a major component of plant adaptation. *Fanomics* is a term used to refer to the combination of genomics with analysis of trait stability under stress in order to understand mechanisms underlying canalization or robustness. We explore the phenotypic stability of homozygous wild barley (*Hordeum spontaneum*; The B1K collection) ecotypes and their hybrids in the face of environmental stress. Stability of a certain genotype is defined as the relative change in the trait variation between individuals and calculated as index of Fano actor or coefficient of variation (CV). This is compared with high resolution genotype and proteome data to identify potential causal diversity affecting canalization and the mechanism underlying. Correlation analysis between genome-wide heterozygosity and phenotypic stability under both environments shows no correlation for any of the vegetative or reproductive traits. However, genetic association analysis between high-throughput gene-based single nucleotide polymorphism (SNP) data and canalization against water deficit data reveals several significant loci which predominantly reduce rather than improve canalization in hybrids (i.e., decanalization). Comparative proteomic analysis between different B1K accessions grown under temperature stress identifies differential expression of cryptic peptides. We currently integrate these phenotypic, proteomics and new exome-seq datasets to explore characteristics of DNA and protein diversity and its possible causal effects on plant robustness to environmental perturbations.

The European *Avena* database and data acquisition from geographically distributed characterization and evaluation projects

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The European *Avena* Database (http://eadb.jki.bund.de) is one of the Central Crop Databases held within the European Cooperative Programme for Plant Genetic Resources (ECPGR). It integrates passport data for 32.657 oat genetic resources accessions with characterization and evaluation (phenotyping) data, pictures and limited allele information for parts of the collected accessions. It allows for combined research on passport, characterisation and evaluation data.

During targeted actions funded by EU DG AGRI based on the regulations EC 1467/94 and 870/2004 a comprehensive information and documentation system for phenotyping data, including background information on field experiments and design, experiment sites, observation methodology and standard cultivars has been set up. Web tools for the online management of characterization and evaluation of genetic resources in geographically distributed field experiments have been developed (http://aveqtest.jki.bund.de). They cover the management of a working collection, randomization of field plans, generation of downloadable field books and electronic spreadsheets for data acquisition in field and laboratory, and the import of spreadsheet data, pictures and picture metadata.

Currently 163 characterization and evaluation descriptors (traits), described with 385 different measuring, counting or scoring methods are available in the database. There is no limited set of descriptors and methods given by the database structure. Any new trait or observation methodology can be added easily and methodological details, including the textual decoding of scores, can be precisely documented online in the database. In total nearly 4000 accessions have phenotyping data and for more than 500 accessions pictures are available. Phenotyping data cover disease resistance, tolerance to abiotic stress, phenological and morphological data, and parameters of technical and nutritional quality, e.g. contents of protein, fat, dietary fiber and antioxidants.

Beneath the poster an online demonstration will be given on querying and retrieving results from the EADB, on the generation of field plans and field books for phenotyping in field experiments, on mapping of observation data in Excel sheets to the database structures and on import and archiving of Excel sheets and pictures with the project management web tools.

Identification of oat accessions by means of reverse phase high performance liquid chromatografy

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The introduction of oats into the gluten-free diet has been a topic of debate in recent years as some works suggest that the immunogenicity of oats depend on the cultivar used. Varietal identification of oats has been clearly demonstrated by RP-HPLC. Moreover, it has been speculated that the identification by this technique of patterns related to CD toxicity would be useful to select oat varieties with a reduced immunogenicity.

We have analyzed the peak patterns of the alcohol soluble fraction of a collection of 132 accessions and cultivars of *Avena sativa* L. from Mediterranean area and the USA by RP-HPLC. A variable number of major reproducible peaks eluted in the hydrophobic range (33–43 min) were identified for each accession (seven peaks in average). The utility of RP-HPLC to determine relationships among genotypes and for identification of oat accessions, as well as the association between the presence of certain peaks or patterns and toxicity for celiac patients, are discussed.

Improvement of anther culture for doubled haploid line production in wheat (*Triticum aestivum*) using omics-based techniques

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Wheat (*Triticum aestivum*) is the third most important crop worldwide and the most important one in Europe. Breeding of new wheat cultivars can be accelerated by 2-3 years when using doubled haploid (DH-) lines instead of conventional line breeding. Adaption to changing climatic environments, safeguarding the global needs of food for a growing population, and exploitation of new and up to now unsuitable growing areas via better stress resistance of new cultivars will faster become possible.

The goal of our work is to unravel the developmental pathways underlying androgenic microspore development during anther culture of wheat. This knowledge shall be used to increase the efficiency of doubled haploid wheat production, especially to develop genotype independent efficient anther and/or microspore protocols. The gained knowledge can also be used to improve doubled line production in wheat relatives, such as emmer (*Triticum diccocum*), durum wheat (*Triticum durum*) and spelt wheat (*Triticum aestivum* subsp. *spelta*).

Microspores of three developmental stages from *Triticum aestivum* will be manually selected from developing anther cultures and probed at the RNA- and metabolite-level. Additionally, QTL analysis will be made with a segregating DH population. In combining results of all three regulatory mechanisms, we hope to facilitate a knowledge-based improvement of wheat anther culture. Here, first results of our work will be presented.

Using gene pool of synthetic wheats for increasing of common wheat biodiversity

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The small genetic variability in most crop plants, including that of common wheat, *Triticum aestivum* L., can limit the advancement of crop breeding. Relate wheat species, as a rule, cannot be used directly as synthetic breeding donors and transgression of target traits is possible by means of special procedures. The main prerequisite of successful introgression of genetic material in the tribe of *Triticeae* Dum. is a potential possibility of intra- and intergeneric crosses conditioned by close philogenetic relationship for most of the species. Common wheat is most frequently used as a recipient. Disease and pest resistance and salinity tolerance are introgressed into it, although many species of the tribe *Triticeae* are potential donors of other agronomic traits.

We suppose that producing artificial (synthetic) wheat amphiploids is an easy way to utilize the useful genes of related species. Nowadays the attempts of using wheat synthetics for breeding are numerous. Some of studied hexaploid wheat synthetics have complex immunity to fungi disease. For example, *Triticum kiharae* Dorof. et Migusch.

Flow cytometric analysis of cell cycle in long-term stored seeds: A new method for assessing seed viability?

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The purpose of this study is to establish a fast and accurate screening method for determining seed viability stored in the genebanks using flow cytometry.

After mitotic division cells undergo the first growth phase (G1), then the phase of DNA synthesis and the second phase of growth (G2). Diploid cells in the G1 phase in nucleus contain DNA 2C and G2 - 4C. Flow cytometry allows determining the quantitative ratio between cells which are in different stages of the cell cycle (G2/G1), which provides information on the physiology of seed, its stage of development, maturity, advancement of germination. G2/G1 ratio was considered to be a marker of germination, used to track the processes of seed conditioning: in conditioned seeds is higher.

Thus, the question is: Can the proportion of cells in different cell cycle phases be used to analyze of seed aging process in a long-term storage?

Preliminary experiments were carried out with rye kernels subjected to different methods and time of storage. The results show differences in amount of cell in sub G1 phase according to methods and time of kernels storage. It seems to be a marker of seed viability.

Timing and fate of floral development in wheat

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Grain number, which is one key factor for wheat (*Triticum aestivum* L.) yield, is determined by floral development and abortion. However, the floral development and abortion process is not yet clearly understood. To study this process in great detail, thirty European hexaploid winter wheat cultivars were grown for dissection work under both greenhouse and field conditions. Dissections were carried out mainly following the Kirby scale and the corresponding developmental stages according to the Waddington and Zadoks scales were also recorded. A developmental model was derived to explain the observed floral development and abortion process. According to our model, floret primordia and floret number develop their maxima at green anther and yellow anther stages, respectively. Floret fertility is primarily impaired by two degradation processes. Floret primordia arrest and abort, but more mature florets also abort. Visible floret primordia abortion is a sudden event and generally occurs during the interval from the tipping to heading stages (2-4 days). More mature floret abortion, however, is a gradual process which happens during different stages of development, and is most likely triggered by competition effects between florets within a given spikelet. Finally, we show that macroscopic appearance (Zadoks scale) and floral development (Kirby and Waddington scales) of the basal floret of the central spikelet are linked, and that floral development can generally be assessed based on external morphology without destructive dissection work.

Genetic progress in the Romanian *Triticale* breeding program

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Triticale is cultivated in Romania mainly on the hilly regions around Carpathian Mountains, on the acid soils, where covers about 1.5 million ha. Triticale is currently cultivated on 100 to 150 thousand ha, representing 1.5% from the total arable land. Since 1971 when the triticale breeding program has been initiated, 14 varieties have been registered. The Romanian triticale germplasm was developed based on (i) primary triticale, (ii) crosses with bread wheat and by germplasm exchanges with important foreigner programs in winter or spring types.

Genetic progress for yield estimated over 29 years since the first variety has been registered (1984), raised up to 43 kg / ha^{1} and year^{1} or 0.74 % /year^{1}, value more or less similar with those realized in others important triticale breeding programs in the world. Yield enhancement has been achieved mainly by: improving fertility of spikes, plumpness of kernel, the test weight and introduction of short straw *RhtB1b (Rht1)* and *Ddw1 (Hl)* genes. Following the long term selection pressure under artificial conditions, for biotic and abiotic stresses, yield stability of the new released varieties over a large variability of environments increase constantly. As result of the continuous pressure of selection for resistance to leaf rust, septoria leaf blotch, Fusarium head blight (FHB) and Barley yellow dwarf virus (BYDV), the most frequent and most damaging diseases under climate conditions of Romania, a triticale germplasm with higher level of resistance has been developed. Most of recently registered varieties possess mainly partial resistance against leaf rust, while resistance to BYDY it is conferred by at least two sources of resistance. Regarding FHB there is a good level of resistance Type II (spreading of disease into spikes), but in contrast to wheat not closely related with accumulation of associated toxic compounds (mycotoxins) in kernels that could be more frequent rather high and a real threat to food safety.

Chasing a new flowering time gene

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Bread wheat (*Triticum aestivum* L.) is a staple food of 40% of the world's population. Population growth and extreme weather fluctuations call for development of new cultivars resistant to environmental stress and with increased and more stable yields. The yield can be modified by a number of ways; one of them being the optimisation of flowering time (FT) to local climatic conditions.

Apart from major FT genes, which have already been determined, there are minor genes, some of which have been identified as QTL´s. Most of them have not been discovered yet and although our current knowledge of pathways regulating FT is improving, it remains far from complete. Clearly, there is a need to identify alleles and genes which may be useful in wheat improvement.

The main objective of our work is to localise a new FT gene. We have identified 12-day difference in heading time between wheat varieties Paragon and Kaerntner Frueher. There were no differences in the main FT genes (*Vrn-A1a*, *Vrn-B1c*, *vrn-D1*, *vrn-B3* and *Ppd-D1b*) between these two varieties except dissimilar alleles of *Rht8* gene (*Rht8a* and *Rht8g*). However, this variation should not cause a large difference in FT. Thus, we hypothesise that there may be a novel gene/allele involved. We observed a temperature-dependent effect of the hypothetic gene in field as well as in controlled conditions (16/8h, 20/16˚C), when lower temperature prolonged FT difference up to 28 and 30 days in field and growth room, respectively.

In order to localise the source of this interesting phenotype, both lines were crossed and F_2 mapping population has been developed. A preliminary localisation of this gene will be done using DArT analysis, which will be followed by QTL analysis. As there are typically 2,000-3,000 DArT polymorphism identified within a wheat genome, we expect that the high marker density will permit identification of the locus involved.

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Mirza Gökgöl – A pioneer of wheat genetic resources from Turkey

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Mirza Gökgöl (1897–1982), a leading plant scientist in the 20th century, was born as Mirza Hacızade in Elisabethpol (present-day Ganja), Azerbaijan. He studied agriculture under Erwin Baur in Berlin and made his PhD in the 1920s. In 1926, he founded one of the first Turkish Plant Research and Experiment Stations in Yeşilköy near Istanbul, which later became the Yeşilköy Institute of Research and Experimenting. He was director of this institute until his retirement in 1961. His scientific interest focused on wheat, but he also worked on various aspects of other crop plants in Turkey. During 1929–1955, he collected and intensively studied landraces of wheat, barley, lentil and their wild relatives, among them ca. 20,000 accessions of various cultivated wheat species from all over Turkey; however, this collection was unfortunately lost after his retirement. Several thousand herbarium specimens, mainly of *Triticum*, are preserved in AARI, Menemen, Izmir. He described numerous new botanical varieties (although many not validly published), and wrote about wheat domestication and origins of wheat cultivation. He considered that wheat diversity in Turkey was so rich that there was no need for Turkish breeders to utilize foreign material in their breeding programmes. Since his ample work published in numerous books and scientific articles mainly in Turkish and German has not been accessible by the scientific society today, even in Turkey, we intend to prepare an overview of his work. His views and achievements will be presented in comparison with present-day knowledge. Gögköl's work is largely forgotten in Turkey, and he is not well-known abroad.

Association mapping in bread wheat: The case of an Italian collection

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Association mapping, a high-resolution method for mapping quantitative trait loci based on linkage disequilibrium, holds great promise for the dissection of complex genetic traits, like most of those of agricultural importance. A genome-wide association study (GWAS) is in progress on a *Triticum aestivum* germplasm collection consisting of 184 lines (landraces, old and modern varieties), summarizing the process of wheat breeding in Italy during the last hundred years. The collection has been genotyped with the Infinium iSelectHD 90K (Illumina). SNP data were filtered using the R-Studio software (http://www.rstudio.com/). Genotypes with more than 10% of missing data, monomorphic and rare SNPs (Minor allele frequency < 1%) as well as markers with more than 5% of missing data were not considered for the population structure analyses performed on PAST (PAlaeontological STatistics) software (http://folk.uio.no/ohammer/past/). Out of 81,587 available SNPs, 23,329 were used for phylogenetic cluster analysis and Principal Coordinate Analysis (PCO). The landraces were clearly clustered together, but no other groups could be discriminated. This trend was also observed when the same SNPs dataset was employed for the analysis of the population structure with the *Structure* software (http://pritchardlab.stanford.edu/structure.html). Even if the germplasm collection summarizes a century of the bread wheat breeding in Italy, modern varieties did not appear stratified. The lines were grown for two growing seasons in two locations in Italy and assessed for several agronomic, morphologic and qualitative traits. Significant differences for all the parameters were observed: a clear trend from old to new varieties was noticed, leading towards earliness, plant height reduction, kernel weight decrease, kernel hardness increase and protein content reduction (coupled, nevertheless, with an improvement in protein quality). The association analysis for the considered traits is under way.

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The spectrum of polymorphisms observed in several populations of barley chloroplast mutator plants resembles the hallmark of a defective gene involved in plastome mismatch repair

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The barley chloroplast mutator was described as a mutant in a nuclear gene (C*pm/cpm*) that when homozygous produces a wide spectrum of cytoplasmically inherited chlorophyll types, but it was not observed inducing inheritable sterility. Recent investigations through a TILLING approach applied on seedlings from two families carrying the homozygous mutator genotype during 12 to 17 generations of natural auto-pollination, showed a wide range of plastome sites affected mostly by point mutations. In the present work, in order to better evaluate this mutator, we anticipated the analysis to the F_6 generation. The new results coming from four different families showed a similar landscape to that previously observed in more advanced generations. A wide range of plastome sites was affected by substitutions and by small indels of 1 or 2 bp located in microsats. Interestingly, we also confirmed the presence of large indels characterized by carrying direct repeats in the extremes of the deleted/inserted fragments and, furthermore, a high frequency of different combinations of five polymorphisms affecting the *rpl23* gene. Both facts strongly suggest that there is an augmented homologous recombination in the plastome of mutator plants. All the data show the barley mutator as a very powerful tool to broaden the otherwise scarce plastid genome variability and indicate that the molecular detection by CJE digestion of mutator induced plastome mutants can start at least in the F_6 generation. Altogether, previous and present results, suggests that the spectrum observed is the hallmark of a defective mismatch repair gene, which at least acts on the plastome of barley plants. A few defective mismatch repair genes were previously studied in higher plants, but they act on nuclear and/or mitochondrial genes and none of them were observed so highly active on the plastome.

Grain yield and nitrogen use efficiency in Bulgarian bread wheat cultivars grown at contrasting N levels

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Nitrogen (N) is the major nutrient that limits crop production. The yield increments in cereals over the past 50 years have been associated with substantial increase in the global consumption of N fertilizers. In wheat, it is mostly due to the introduction of modern semi-dwarf N-responsive and high-yielding cultivars. Nowadays, in consonance with the market requirements for energy efficiency, public awareness on the environmental consequences of N loss, and the raised consumer demands for healthier products, the EU follows the 91/676/CEE Nitrates Directive encouraging the reduced use of N fertilizers while maintaining acceptable grain yield. These objectives can be met by developing and growing of cultivars with better nitrogen use efficiency (NUE). In the present study, a diverse collection of 100 modern and old cultivars, breeding lines, and *Rht* near-isogenic lines, released in Bulgaria was used to assess the genetic variation in yield and NUE in response to contrasting N supply. The plant material was grown at two input rates (N_0 and N_{12}) in three environments of different soil type and microclimate (Sofia, General Toshevo and Sadovo) using partially randomized block design. The following traits were measured: grain yield and yield components, plant height, and earliness. The agronomic NUE, defined as grain yield increase and total grain N accumulation per fertilizer N supply was obtained in part of the material. The effects of nutrient level, genotype and environment were determined. The modern and old cultivars were compared by their response to N application.

12C01, a heritable mutant with multi-trait variations from 60Co radiation in wheat (*T. aestivum*)

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4 years research showed 12C01 was a heritable mutant with multi-trait variations (MTV) from 60Co radiation in wheat (Tr. aestivum). Superior MTV was observed from its descendant of M2-M4 deduced it could be used for super-high yield breeding research. Wild type (WT) Shi 4185, an elite genotype used as check cultivar in regional yield testing in Hebei province, was treated by 60Coγ ray of 250Gy in 2010. A mutant with positive MTV, named 12C01, was selected from the M2 generation in 2012.

Phenotypically it performed vigorous with more spikes/plant (SP), longer spikes, more spikelets and kernels, bigger kernel size and thicken stem. SP, Spike length/main stem (SLMS), spikelets/main stem (SMS), kernels/main stem (KMS) and thousand-kernel-weight (TKW) were 11, 12.1, 26, 92, 52.84, compared with the WT, they were 22.22%, 37.50%, 18.18%, 31.43% and 36.33% advantages, respectively. Internode diameters (ID) of 1st, 2nd, 3rd, 4th, 5th and 6th were 0.43cm, 0.47cm, 0.5cm, 0.53cm, 0.55cm and 0.41cm demonstrated 30.30%, 23.68%, 21.95%, 23.26%, 19.57% and 17.14% advantages than WT. Thickness of it were 0.06cm, 0.07cm, 0.08cm, 0.09cm, 0.12cm and 0.15cm which were 20.00%, 0, 14.29%, 12.50%, 9.09% and 15.38% over WT, respectively.

Contrasted to normal theory of radiation, traits were seriously segregated in its M_3. Recording from a population of 249 plants, Plant height (PH), SP, SLMS, SMS, KMS and TKW were 58-61cm, 5-16cm, 8.3-13, 17-23, 38-91, 33.33-48.65g, comparing with WT they were ranged -7.2-29.6%, -44.44-77.77%, 0-56.63%, -1.53-21.05%, -44.12-33.82% and -4.91-38.80% individually. A promising one, named 13C01, was selected in 2013. Characteristics of PH, SP, SLMS, SMS, KMS and TKW were 74.3cm, 12, 12.7cm, 22, 91 and 48.65g, variations extent were 11.90%, 33.33%, 53.01%, 15.79%, 33.82% and 38.80%, and ID for 1-5 were 50.00%, 35.00%, 24.44%, 38.64% and 13.51%, respectively. Meanwhile another 4 ones were selected from added generation of M_4, SP, LSMS, SMS, KMS and TKW were 10%, 23.53-47.06%, 19-50%, 23.33-55% and 34.04-38.25% advantages than WT. This year 79 M_5 lines from added generation are under yield testing in plots, 79 M_4 lines and 31 M_5 lines from normal season selecting and from added one individually are under further selection.

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HvFT1 polymorphism and effect – survey of barley germplasm and expression analysis

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Flowering time in plants is a tightly regulated process. In barley (*Hordeum vulgare* L.), *HvFT1*, homolog of *FLOWERING LOCUS T*, is the main integrator of the photoperiod and vernalization signals leading to the transition from vegetative to reproductive state of the plant. This gene presents sequence polymorphisms affecting flowering time in the first intron and in the promoter. Recently, copy number variation (CNV) has been described for this gene. An allele with more than one copy was linked to higher gene expression, earlier flowering, and an overriding effect of the vernalization mechanism. This study aims at i) surveying the distribution of *HvFT1* polymorphisms across barley germplasm and ii) confirming whether CNV is related to phenotype.

We analyzed *HvFT1* CNV in 108 winter, spring and facultative barley varieties. There was more than one copy of the gene (2-5) only in spring or facultative barleys, i.e., without a functional vernalization *VRNH2* allele. CNV was investigated in several regions inside and around *HvFT1*. Two models of the gene were found: one with the same number of promoters and transcribed regions, and another with one promoter and variable number of transcribed regions. This last model was found in Nordic barleys only.

Under long day conditions the earliest flowering lines were those with a sensitive *PpdH1* allele, which induced earlier *HvFT1* expression and flowered before than typical spring cultivars (with recessive *ppdH1*), independently of *HvFT1* copy number. *HvCEN* also affected largely flowering time, although independently of *HvFT1* expression. A possible interaction between them is discussed. Among spring cultivars with different number of copies, no clear relation was found between CNV, gene expression and flowering time. This was confirmed in a set of double haploid lines of a population segregating for *HvFT1* CNV. Earlier flowering in the presence of several copies of *HvFT1* was only seen in cultivar Tammi, which carries one promoter, suggesting a relation of gene structure with its regulation.

Genotyping-by-Sequencing (GBS) of a set of diverse spring barley (*Hordeum vulgare*) accessions

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Next-generation sequencing technologies have become affordable to use sequencing for standard genotyping in genetic mapping studies. The aim of the work presented was the saturation of a set of 192 spring barley accessions with a high density of SNP markers in a Genotyping-by-Sequencing (GBS) approach. The set of barley accessions from different origins represents a broad spectrum of the genetic diversity and exhibits low linkage disequilibrium making it useful for high-resolution association mapping studies. Indeed, the set of barley accessions has previously been genotyped with 45 SSR markers, 1536 Illumina GoldenGate markers, 1935 DArT markers and the 9k iSelect chip. However, a higher number of SNP markers is needed at a density that reflects genome-wide linkage disequilibrium structure and haplotype diversity. The GBS experiment comprised the construction of a reduced-representation 192-plex library and its sequencing on one Illumina HiSeq 2000 lane (1 x 100 cycles). A reference-based computational pipeline was applied to analyze 20 GB of sequencing data. Trimmed reads were mapped against the whole-genome shotgun assembly of the barley cultivar Morex. Increasing the stringency of the criteria for filtering genotype calls reduced the number of loci useful for genotyping from 55,839 to 5,658 biallelic SNPs. In a next step, these SNP markers are to be mapped employing marker position information resulting from Morex contigs anchored to the integrated physical and genetic map of barley. It is expected that the GBS markers can be utilized together with phenotypic data currently gained, e.g. for drought stress response, for the mapping of QTL in whole genome association scans and for linking corresponding QTL to candidate genes.

Genetic diversity in Southern Italy durum wheat old accessions and modern varieties

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The Italian long-term history of durum wheat breeding has been characterized by the constant release of leading varieties that in turn became progenitors of new varieties, selected to perform well under intensive crop management. These events have altered the original genetic structure and genetic diversity pattern of wheat and a large amount of genetic diversity has been lost, reducing the potential for wheat improvement in modern agricultural systems.

"Ex situ" collections that include a considerable amount of genetic diversity and useful phenological, morphological, abiotic, biotic and quality traits might contain traits required for organic, low-input agricultural systems that are not available in varieties selected for modern, high-input conditions. Thus, a comprehensive characterization of germplasm collections is a prerequisite for understanding the extent of genetic variability in the available germplasm and to make it available for breeding needs.

In this study 145 durum wheat accessions collected in Southern Italy since 1947 and now preserved "ex situ" in genebanks, and 28 of the most cultivated varieties in Italy during the past decades, were characterized. To describe the genetic diversity of the whole collection morphological traits (heading date, plant height, spike length, number of spikelet per spike, number of seeds per spike and weight of 1000 seeds), grain quality parameters (umidity, grain protein content, sds and carotenoid content) and 30 nuSSRs markers were used. The data collected were processed by multivariate methods and Bayesian approach.

The joint analysis of the results allowed an evaluation of the genetic diversity between old accessions and modern varieties. This germplasm represents a valuable genetic resource for low-input agriculture breeding programs.

Effect of chromosome 7Hch from *Hordeum chilense* Roem. et Schultz. on carotenoid content and lutein esterification in common wheat

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Colour is an important quality criterion for most wheat end-use products. Yellow colour has become an important selection criterion in durum wheat (*Triticum turgidum* spp. *durum*, 2n=4x=28, AABB) due to its importance for the production in pasta. On the contrary, white flour is traditionally preferred by consumers for the bread-making using common wheat (*T. aestivum* L. 2n=6x=42, AABBDD). However, lutein also contributes in part to the yellow colour of yellow alkaline noodles which is promoting the development of high lutein materials in Australia. Similarly, new end-use products using either einkorn (*T. monoccocum* L. ssp. *monococcum* L., 2n=2x=14, AA), or the new cereal tritordeum (×*Tritordeum* Ascherson et Graebner, 2x=6x=42, AABBH^{ch}H^{ch}) are partly yellow due to the high carotenoid content of these species compared to common wheat.

Hexaploid tritordeum is the amphiploid derived from the cross between the wild barley (*Hordeum chilense* Roem et Schultz., 2n=2x=14) and durum wheat. The addition of chromosome 7H^{ch} to common wheat results in the increase of yellow pigment content. However, the effect of this chromosome in wheat has not been tested in euploid combinations. In this work, we have used five genotypes of common wheat with either translocations or substitutions involving the chromosome 7H^{ch}. These lines were characterized using molecular markers and further confirmed using FISH (fluorescent in-situ hybridization). These lines were analyzed for carotenoid content at harvest. Chromosome substitution lines of *H. chilense* into 'Chinese Spring' wheat background available from other sources were used as controls along with durum wheat 'Kofa' and tritordeum 'HT621'. All the lines carrying *Psy1* from *H. chilense* had a higher seed carotenoid content (mainly lutein) than the wheat control. However, the profile of lutein esterification differed among the lines and it depended on the interaction with the wheat genes. This research was supported by Grants AGL2011-24399 and AGL2010-14850 from the Ministerio de Economía y Competitividad (MINECO), including FEDER funding. MGM was recipient of a predoctoral grant BES-2012-055961, also from MINECO).

Deletion mutation of Wheat *PHYTOCLOCK 1* induced by heavy-ion beam irradiation leads to the decreased photoperiodic sensitivity and extra early-flowering phenotype

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Four extra early-flowering mutants, named *extra early-flowering1* (*exe1*), *exe2*, *exe3*, and *exe4*, were identified in *Triticum monococcum* strain KU-104-1 following heavy-ion beam mutagenesis. Among them, *exe1* and *exe3* have the deletion mutation of a circadian clock regulator gene *Wheat PHYTOCLOCK 1* (*WPCL1*). The *exe* mutants showed early heading-time about 30 days earlier than WT plants in the field. Analysis of plant development in a growth chamber showed that the speed of leaf emergence was accelerated in *exe* mutants at the reproductive stage compared to wild-type (WT) plants, and the number of leaves was reduced in *exe* mutants. Furthermore, the *exe* mutants have a decreased photoperiod sensitivity compared to WT plants; this difference is in line with the differences in leaf numbers under LD or SD conditions between *exe* mutants and WT plants. Analysis of *VERNALIZATION 1* (*VRN1*), a flowering promoter gene, showed that it was more highly expressed in seedlings at early developmental stages in *exe* mutants under short day conditions as well as long day conditions. Expression analyses of clock related genes such as *LHY*, *FKF1* and *TOC1*, indicated that the expression patterns of the clock related genes in *exe* mutants were different from those in WT plants especially under short day conditions. These findings suggest that the deletion of *WPCL1* induces disruption of the short day response, resulting in the decreased photoperiodic sensitivity and extra early-flowering phenotype in *exe* mutants.

Elevated Partial Pressure of Oxygen (EPPO) – Identification of seed ageing mechanism in a short period

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The EPPO, short for Elevated Partial Pressure of Oxygen, methode was developed by Groot et al. (2012, Annals of Botany) and uses the possibility to accelerated seed deterioration under dry conditions instead of the common method to apply high humidity and high temperature. Interestingly, the seed treatment with high-pressure oxygen triggers similar morphological ageing symptoms as are usually observed after long-term dry storage.

To challenge the comparability of the EPPO method we tested the progeny of the Oregon Wolfe Barley (OWB) mapping population on germination after high-oxygen pressure treatment at 40% relative humidity for 9 weeks. The analysed data were compared with results obtained in 2009 (Nagel et al., Euphytica). By application of controlled deterioration and accelerated seed ageing, three major QTL (quantitative trait loci) on chromosomes 2H, 5H and 7H were revealed. Analyses of available datasets using recently published Restriction Site Associated DNA (RAD) linkage map should provide unique information about the comparability of experimental seed ageing methods.

The EcoSeed project – seed performance in a changing climate

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Seed quality is of paramount importance to agriculture, food security and the conservation of wild species. Considerable economic losses result from sub-optimal seed performance, undermining food security and livelihoods. Seed quality is strongly influenced by the environmental stresses experienced by the mother plant. Climate change will further exacerbate economic losses and decrease the predictability of seed yield and quality for the farmer. The looming challenges of climate change and food security require new knowledge of how stress impacts on seed quality, as well as a re-appraisal of optimal storage conditions. EcoSeed addresses these challenges by bringing together a group of distinguished European experts in seed science and converging sciences to characterise seed quality and resilience to perturbation. EcoSeed combines state-of the-art "omics", epigenetics, and post-"omics" approaches, such as nuclear and chromatin compaction, DNA repair, oxidative and post-translational modifications to macromolecules, to define regulatory switchboards that underpin the seed phenotype. Special emphasis is placed on the stress signalling hub that determines seed fate from development, through storage, germination and seedling development, with a particular focus on seed after-ripening, vigour, viability and storability. Translation of new knowledge gained in model to crop and wild species is an integral feature of EcoSeed project design, which will create a step-change in our understanding of the regulatory switchboards that determine seed fate. Novel markers for seed quality and new "omics" information generated in this project will assist plant breeders, advise the seed trade and conservationists alike. In this way, EcoSeed will not only be proactive in finding solutions to problems of ensuring seed quality and storability but also play a leading role in enabling associated industries to better capture current and emerging markets.

Ecogeographical analysis of barley landraces from Spain reveals differences associated to plant adaptation

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The Spanish barley core collection constitutes a representative group of inbred lines derived from landraces cultivated in the Iberian Peninsula until the second half of the 20th century. After curation to remove possible duplicates, the collection now has 138 landrace-derived inbred lines, and 16 cultivars. The collection has been analysed with the 9K iSelect SNP genotyping platform, and with the platform DArTseq®, a genotyping-by-sequencing (GBS) system. After curation and alignment to the barley physical map, a total of 9779 SNPs with map position have been kept for further analysis, 6223 from the iSelect chip and 3556 from GBS. A bayesian clustering procedure (STRUCTURE) produced four clusters of genotypes, or populations. Two of them, the largest ones with 49 and 67 individuals, corresponded largely to Spanish 6-row landraces. These two groups were distributed over the country following clearly distinguishable climate patterns, indicating differential adaptation. In this study, we present a survey of the genetic diversity and genetic differentiation of these two groups of landraces, using softwares Arlequin and Bayescan to determine locus heterozygosity and F_{ST}, and the detection of outlier loci that may indicate divergence between the populations. Also, the relationship of the distribution of alleles vs geographical distance has been analysed using Genalex. Loci most linked with geographical distance and that best differentiate between the populations indicate regions that have been likely targets of selection for adaptation to the specific climates and other environmental constraints.

Evaluation of anatomical traits of the root cross section in durum wheat cultivars

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Anatomical root traits are defined by the internal structure of the plant, and the arrangement of cells and tissues. Field-grown plants of ten durum wheat cultivars were investigated for variation in anatomical traits of the root cross section. Two plants were grown per genotype, and two roots were collected for each plant. Sections of tissue segment were selected 0-3 cm from the base of the root. The microscope was fitted with Video Camera Module (NIKON, DS Camera Head DS-5M) and images were analysed with the program *RootScan*. The anatomical root traits were collected from the root cross section area, aerenchyma lacunae, stele and xylem vessel area. Analysis of variance showed significant differences among genotypes for root cross-sectional area, root-sectional cell wall area, total cortical area, cortex cell wall area, number of lacunae and stele cell wall area. No significant differences among cultivars were detected for the total number of xylem vessels in stele region. Aerenchyma forms due to root cortex programmed death of parenchyma cells, resulting in replacement of cells with air-filled channels called lacunae. Highly significant differences (P <0.01) among genotypes were detected for this trait with cv. Cappelli showing the highest number of lacunae. Many anatomical root traits were significantly correlated. In particular, the total number of lacunae was negatively correlated with the number of cell files, cortex cell count and area of cells in the cortex.

Analysis of chemical growth regulator influence on gibberellins biosynthesis pathway genes expression in common wheat genotypes containing dwarfing genes

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The aim of examinations was estimation of commonly used growth regulator - chlormequat chloride (CCC) application on expression of selected genes involved in bioactive gibberellins biosynthesis pathway in common wheat plants, containing different dwarfing genes. The material comprised isogenic lines of common wheat cv. Bezostaya 1 with *Rht-B1b* (GA-insensitive) and *Rht12* (GA-sensitive) dwarfing genes and tall control form. Seven-day-old seedlings of analyzed lines were sprayed with CCC solution. Three days after application the leaves were collected and total RNA was extracted using Trizol reagent. One microgram of each RNA sample was reverse transcribed to cDNA. Obtained cDNA (80 ng for each sample) was used as a template in qPCR.

Chlormequat chloride application caused up-regulation of ent-copalyl diphosphate synthase (CDS) expression. This observation can confirm feedback regulation of bioactive gibberellins synthesis in plant tissue. Similar results were observed for all analyzed lines regardless of dwarfing genes presence. Analysis of results for genes encoding GA20-oxidase (GA20ox) and GA3-oxidase (GA3ox), which are key enzymes in transformation of precursors into biologically active gibberellins, revealed that growth regulator treatment caused mainly down-regulation of their expression. For GA20ox gene about twofold decrease of transcript amount after CCC treatment was observed for all analyzed lines. Expression of GA3ox gene was down-regulated in comparison to respective control forms for tall line and *Rht12* containing line. However, for Bezostaya 1 *Rht-B1b* line, an increase of transcript level was noticed after CCC application. In presented research analysis of expression of GA2-oxidase (GA2ox) gene were also carried out. GA2ox is a major enzyme caused transformation of bioactive GAs into forms without biological activity. Analysis of GA2ox gene showed that in tall line growth regulator activity caused sixfold increase of expression, whereas for lines containing dwarfing genes twofold decrease of transcript level was noticed.

Our results confirmed that application of chemical growth regulators influence on genetic mechanism regulating bioactive GAs biosynthesis pathway. Moreover, we suggest that pattern of response is dependent on dwarfing genes presence in common wheat genome.

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Comparative analysis of *Avena* genus hexaploid species using REMAP and ISSR methods

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Taxonomic relationships in *Avena* genus are not evident. Among others, it is being speculated that *Avena fatua* L. might have originated either from *Avena sterilis* L. or *Avena sativa* L. Alternatively, it could have evolved independently during formation of the hexaploid form. It is being suggested that *A. fatua* may have different A genome than the other well recognized oat hexaploids such as *A. sterilis* and *A sativa*. Studies performed with BARE-1 specific markers demonstrated that this transposon is present in low number copies in C genome while is abundant in the A, B and D once. The observed differences rise the opportunity for analysing putative origin of *A. fatua* and its relationships with other hexaploids. Two marker systems were applied. REMAP with selective primers directed towards BARE-1 sequences in order to get evidences that A genome of *A. fatua* could have different origin than in case of the other hexaploids. ISSR to screen for the whole genome polymorphisms among analysed materials. Numerous parameters (Shannon's index, Percentage of polymorphic loci, Polymorphic information content, AMOVA) were evaluated. The genetic relationships were demonstrated based on hierarchical analysis (UPGMA, PCoA). The REMAP and ISSR platforms were compared by means of Mantel test using genetic distance matrixes.

REMAP and ISSR generated comparable results. Lower variation among wild *A. fatua* samples in comparison to cultivated *A. sativa* seems to be surprising as selection pressure should narrow variation of *A. sativa*. Alternatively, higher variation among representants of *A. sativa* could be related to distinct breeding programs and origin (Australia, Germany and Poland). Due to high level of variation exhibited among *A. sterilis* samples it should be considered as a source of valuable traits that could be used for oat improvements.

Moreover, the presented studies failed to demonstrate that *A. fatua* could have originated via cross with a maternal species having different than *A. sterilis* and *A. sativa* A genome. Nevertheless, our results tend to favour the hypothesis that *A. sterilis* could be the progenitor of *A. sativa* and *A. fatua*.

Phenotyping of agronomically relevant traits in a rice germplasm collection under different water management systems for association mapping studies

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Three hundred temperate japonica rice accessions were grown in the field under two water management conditions (flooded and aerobic soil) to evaluate their agronomic performances under water limited conditions. Three replicates for each accession and condition were tested in a randomised block design. An extensive list of agronomical traits related to plant and seed morphology were assessed on five individual plants per each replication, including flowering and maturation time, plant height, chlorophyll and flavonols content, panicle length, grain width and length. Preliminary data analysis highlighted a large variability of the recorded traits among the accessions with a large effect of the aerobic conditions on the agronomic traits. Several accessions were identified as out-performing under aerobic conditions, suggesting that genetic determinants for adaptation to this water management system does exist in the analyzed germplasm panel. In parallel, genotyping of the rice accessions was carried out with a Genotyping-by-Sequencing approach. Genome-wide association studies carried out using Tassel allowed the identification of significant associations between genetic haplotypes and the target traits analysed.

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Gene space organization and evolution of wheat chromosome 1BS

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Physical map of chromosome 1BS was constructed within the framework of the European consortium *TriticeaeGenome*. Fingerprinted BAC clones were assembled by LTC software, into 254 contigs covering 274 Mb (87%) of 1BS. These contigs were re-organized into 57 long scaffolds (average 4.5 Mb, max. 20.8 Mb) covering 260 Mb (83%) of 1BS. In total, 2,438 markers, of which 87.5% are gene related markers, were assigned to 1BS contigs. The anchored physical map comprised of 53 LTC scaffolds covering 243 Mb (77.4%) of 1BS. The consistency observed between BAC-based physical map and gene order of the orthologous regions of model grass species (*Brachpodium*, rice, and sorghum) verifies the reliability of LTC assembly. Anchored 1BS physical map confirms previous conclusions suggesting a general conservation of synteny and colinearity between wheat 1BS and orthologous regions. Transcriptional map of 1BS obtained by hybridization of 1BS MTP pools with NimbleGen 40K unigene microarray showed that the 1BS gene space spans the entire length of the chromosome arm with a two-fold increase of gene density from the centromere to the telomere. In addition, 76% of the genes are organized in small gene islands composed of two to three genes. Our results confirm that 1BS underwent exceptional accumulation of non-syntenic genes. Furthermore, over two-fold increase in the proportion of non-syntenic genes, as well as more than two-fold expansion in size, were found in sub-telomeric and telomeric parts of 1BS as compared with the centromeric part. Comparison of the size of 1BS and its orthologous segment of *Brachypodium* revealed an average of 30-fold expansion in wheat chromosome relative to *Brachypodium* with a non-uniform distribution of expansion along 1BS from the centromere to the telomere. This pattern is in contrast to the relatively uniform expansion previously found in chromosome 3B of wheat and other cereal chromosomes. Our findings suggest that evolutionary forces that shaped the wheat genome may differ between chromosomes of the same genome of this allopolyploid species. The high quality physical map constructed in the current study is providing a solid basis for the assembly of a reference sequence of chromosome 1BS and its breeding applications.

Using NGS-enabled genetics to improve marker selection and design in hexaploid wheat

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The identification of genetic markers linked to important agronomic traits is of vital importance to ensure food security and is an ongoing effort to improve elite lines. We are focused on the resistance to yellow rust conferred by *Yr15* in bread wheat, a hexaploid (2n=6x=42) organism with a highly repetitive genome for which the first draft reference has recently been made available. We designed a methodology based on an F_2 population derived from near isogenic lines, in our particular experiment between Avocet 'S' and Avocet 'S' containing the *Yr15* resistance locus. Total RNA was extracted from leaves from a number of susceptible and resistance plants. We sequenced cDNA libraries generated from the parental lines and six bulks from the segregating F_2: three susceptible and three resistant bulks, as determined by virulence assays. All libraries were barcoded and sequenced in four Illumina Hi-Seq 2000 lanes.

The RNA-Seq reads were aligned to the NCBI wheat Unigene set v60, where the homoeologues are collapsed in a canonical sequence, and to gene models from tetraploid and hexaploid wheat varieties (Krasileva et al, 2013). We called for polymorphisms (SNPs) between the consensuses of the progenitors and calculated the relative frequencies of the putative SNPs between the segregant bulks to obtain the Bulk Frequency Ratios (BFR, Trick et al., 2012). This approach allows us to characterize SNPs and potentially other genomics variants that are not accessible to traditional SNP callers that assume underlying diploid genomes and less allelic variation than the levels found in polyploid grasses.

To prioritize the putative variants we aligned the gene sequences to the Wheat Chromosome-based Survey Sequencing Project coordinated by the International Wheat Genome Sequencing Consortium (IWGSC) and selected the variations that were enriched (BFR > 6) in at least two of the independent bulks and which aligned to the short arm of the chromosome group 1 (location of *Yr15*). We developed KASP assays to validate the SNPs and generated a genetic map, which was improved using the genes with SNPs within the corresponding syntenic homologues in *Hordeum vulgare*. The final genetic map includes SNP markers that are more closely linked than the current markers used for marker assisted selection of *Yr15*.

Creation and validation of the Spanish durum wheat core collection

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Spanish wheat landraces have a considerable polymorphism, containing many unique alleles, relative to other collections. The existence of a core collection is a favored approach for breeders to efficiently explore novel variation and enhance the use of germplasm. In this study, the Spanish durum wheat core collection (CC) was created using a population structure-based method, grouping accessions by subspecies (*dicoccon*, *turgidum* and *durum*) and allocating the number of genotypes among populations according to the diversity of 39 simple sequence repeats (SSR) markers. A CC of 94 genotypes was established which accounted for 17% of the accessions in the entire collection (555 accessions). An alternative core collection (CH), with the same number of genotypes per subspecies and maximizing the coverage of SSR alleles, was assembled with the Core Hunter software. The quality of both core collections was compared with a random core collection (RC) and evaluated using geographic, agromorphological and molecular marker data not previously used in the selection of genotypes. The CC and CH collections shared 73% of the accessions and had a high phenotypic and genetic representativeness much higher than the RC, which validated their sampling strategies. However, some small differences were found between the two core collections. Geographic and agromorphological variation, phenotypic correlations and gliadin alleles of the original collection were more accurately depicted by the CC. Although more SSR alleles were retained by the CH collection (94% versus 91%) diversity arrays technology (DArT) markers revealed that the CC included genotypes less similar, which fits better with breeders' objectives. The results showed that the core collection designed with the stratified method in conjunction with a diversity index combines well the representation of genetic and phenotypic variability. This CC, which includes a broad range of adapted genotypes and maximizes the representativeness of the genetic diversity in the initial collection, could be extremely useful for a more efficient use of genetic resources in durum wheat breeding.

A dense durum wheat x *T. dicoccum* linkage map based on SNP markers for the study of seed morphology

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Durum wheat (*Triticum turgidum* L. var. *durum*) is a crop of great relevance in the Mediterranean basin. A well-saturated genetic map is an important tool for plant breeding and many technologies are available to increase the abundance of molecular markers suitable for genetic analysis. A segregating population of 136 recombinant inbred lines (RILs), derived from the cross between the durum wheat cv. Simeto and the *T. dicoccum* accession Molise sel. Colli, was genotyped with the wheat 90k iSelect Infinium SNP assay. Fifteen percent (12,449) of the SNPs analyzed were polymorphic between the two parents.

A first map was developed based on the data of 9,040 markers. Forty-five linkage groups were obtained which covered all of the 14 chromosomes of the durum wheat genome. The whole map covered 2,879.3 cM, with a mean length of 205.6 cM per chromosome. The number of markers for each chromosome was from 418 (4B) to 978 (2B), with an average of 645.7. The parents of the genetic map differ for a number of traits of agronomic importance for durum wheat, from traits linked to the spike and kernel morphology to the grain yield.

Quantitative trait loci (QTL) analysis was performed for traits related to kernel morphology and seed weight: 35 and 14 QTL were identified respectively, covering most of the chromosomes. The majority of the QTL identified are located on chromosomes 3A, 4A and 7A.

Wild and domesticated relatives of wheat represent a valuable source of alleles useful for improving durum wheat, which can be transferred to the elite varieties by crosses. Therefore the SNP-based Simeto x Molise sel. Colli linkage map represents a useful tool to dissect the genetic basis of traits of agronomic relevance for the genetic improvement of durum wheat via pre-breeding programs.

Karyotype analysis in *Agropyron cristatum*

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The genus *Agropyron*, which belongs to tribe Triticeae, comprises a number of species that may provide novel genes for wheat improvement. *Agropyron cristatum* L. (Gaertn.) is a perennial species of economic importance as forage; it is facultatively allogamic and auto-compatible, showing high crossability with other members of Triticeae. A detailed knowledge of its karyotype is a prerequisite for the identification of chromosomes introgressed into wheat.

We have analyzed the karyotype of a tetraploid *A. cristatum* (2n=2x=28, PPPP) accession PI222957 from Iran. Fluorescence *in situ* hybridization (FISH) with a set of probes showed specific patterns for the majority of homologous chromosome groups. However, variability in the number and position of FISH signals were observed for some homologues in different individuals. The probe pHvG39 containing GAA satellite sequence showed a large signal in the subterminal region of the long arm of one homologous pair, while the 5S rDNA probe showed two to four sites of hybridization. The repetitive sequence pAs1 showed signals mostly in the terminal regions of all chromosomes and it could be used to identify the individual homologous pairs. The terminal location of the pSc119.2 sequence was also detected on ten to fourteen chromosomes. *In situ* hybridization with the 45S rDNA probe revealed eight hybridization sites, located in the terminal position of the short arms of four chromosome pairs.

The results of this work support the view that the tetraploid *A. cristatum* accession has a non-autoploid nature and that the four P genomes have differed by structural changes like reciprocal translocations. Hence this accession could be a segmental allopolyploid. Variability in the number and position of pSc119.2 and 5S signals, and pAs1 patterns, can help to identify chromosomes involved in the structural changes. More work is needed to characterize the four P genomes in the tetrapod accession, and the first step towards this goal is to establish the karyotype of a diploid *A. cristatum*.

Trait analysis and diversity in some wheat landraces and advance breeding lines under drought and heat stress conditions

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Drought is the most common environmental stress affecting about 32 million hectares million hectares under wheat (*Triticum aestivum* L.) cultivation in developing countries and about 60 million hectares in developed countries. Water and heat are the two main causes of drought stress. In India, nearly 80% wheat is cultivated under irrigated conditions, 66% of it receives only partial irrigations, and the remaining 20% is grown under rainfed environments. Wheat yields have been reported to reduce by 50-90% of their irrigated potential by drought in marginal rainfed environments. Heat stress on the other hand is affecting around 13.5 million hectare grown under wheat in India and 36 million hectare in world. High temperatures during grain filling period adversely affect the plant growth, yield, and grain quality. Climate change is set to increase the frequency and severity of environmentally limited production as global warming will cause more frequent extreme temperature events. By 2020, the wheat season in South Asia will face an increase of 1.08 °C in minimum and 1.54 °C in maximum temperature. Development of heat and drought tolerant genotypes is on priority. Utilization of new diversity is essential. Landraces, which have arisen through a combination of natural selection and the selection performed by farmers, have a broader genetic base. They bear desirable characteristics and more stable yield under stress conditions. One hundred and sixty-one wheat genotypes including land races were evaluated in irrigated timely, rainfed timely conditions two hundred and thirty-one in irrigated timely and irrigated late field conditions for their response to drought and heat stress. Variability averaged over traits was highest under rainfed conditions. Grain yield, plant height, and productive tillers were more sensitive and test grain weight as tolerant under drought. Under heat stress grain yield, grain weight, test grain weight and phenological traits were more sensitive. Productive tillers and grain number per spike were identified as important selection parameters for drought and grain weight (per spike and test grain weight) as for heat tolerance. Genotypes adapted to stressed environments or stable over all environments can be used for introgression of the stress tolerance in elite cultivars.

Using ICARDA germplasm for winter barley breeding in Kazakhstan

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Winter barley cultivated in Almaty, Zhambyl and South Kazakhstan regions. For this area registered 10 varieties, including local cvs Bereke 54, Yuzhni Kazakhstanskiy 43, Baisheshek, Tlek, Aidyn. With improvement of this crop breeders are engaged in Krasnovodopad experimental breeding station (Shimkent). Three winter barley varieties (Aziret-114, Sultan 118 and Orta-111) selected from ICARDA nurseries. Variety Horta-111 (GWB 117-77-9-7 //HT1-02) was selected for resistance to diseases, pests and lodging, cold resistance and size of seeds.

Productivity of winter barley collection samples was evaluated in comparison with the varieties of foreign-standards (Radical, Rihane-3, TARM 92) and domestic breeding Bereke 54 cvs (Kazakh Research Institute of Agriculture and Plant Growing).With a highest productivity 4 samples were differed Baluchistan/Covgbar; Robur/Miraj 1; Pirat/Alger/Ceres 326-1-1 and CWB 117-77-9-7/Alpha/Dura, the superior variety-standard of local breeding Bereke 54. The winter barley collection on electrophoretic hordein spectrum was identified, polymorphic patterns were revealed. A high amylose, low-protein, high-starch sample collection were allocated, which are promising for use in the brewing industry.

Among selected forms with a low-value of β-glucan the winter barley samples AI-18 (Tipper/3yea 26.5//1306//yea 17.27), AI-7 (Alpha/Gumhutriyet//Sonate), AI-4 (Pirate/Malta 1-4 3094-2), 59.630 (Alpha/Cum/3/Car/RM 1508/Coss ICBH 92-0328 OAP-IAP-QAP-4AP-OAP) and 28.350 (Sadik 03) at ICARDA germplasm material which tested previously and isolated forms of these.

According to the maximum degree of the properties "Fe content in the grain", and in particular the reproduction conditions of barley varieties samples are highlighted: Baisheshek in 3 reproductions. In general, the specificity of both protein and starch complex, as well as indicators of extractive, filmy and free proline content the genotypes collection were classified into the 3 clusters.On the base of 630 number the malting barley Aydin cvs was created (2013 registered), and 748 (Mal/OWB 753328-5H/11840-76/3 Radical ICBH 92-0690-OAP-7AP-OAP-15AP-OAP) number – is preparing to transfer in to the State Variety Trial.

Now the collaboration is continuing by project CRP 3.6 Dryland Cereals: A global alliance for improving food security, nutrition and economic growth for the world's most vulnerable poor (Dr. Ramesh Verma).

Collection and evaluation of Israeli genetic resources of bulbous barley (*Hordeum bulbosum* L.)

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Wild barley *Hordeum bulbosum* (bulbous barley) is an important genetic resource for the adaption of cultivated barley to climate changes, especially for the improvement of resistance to biotic and abiotic stress. This wild barley belongs to the secondary gene pool of the genus *Hordeum*. Bulbous barley is originated in the Mediterranean area, and eastward to Afghanistan and Tajikistan. This wild barley is well adapted to extreme habitats like deserts or saline places. In the frame of bilateral German-Israeli cooperation a collection trip was carried out in June 2013 with the aim to develop a systematic collection of *H. bulbosum* from most of the natural habitats in Israel to get more detailed information about the genetic diversity of this species and to identify new sources of resistance especially to net blotch and insect-transmitted viruses. Seeds from in a total of 37 collection sites have been harvested. These collection sites belong to geographical different regions with different types of soil along a climatic gradient extending about 200 km from the Negev Desert to the region Galilee and elevation range from -100m to 1100m.. Till now 76 accessions from 21 collection sites have been proved for resistance to net blotch using artificial inoculation by distributing naturally infested straw debris before planting in the field and additionally by detached leaf tests using defined single spore isolates. 70 of these accessions were also proved for resistance to *Barley yellow dwarf virus* (BYDV) by artificial inoculation with viruliferous aphids of the species *Rhopalosiphum padi* (BYDV-PAV) by DAS-ELISA. 36 accessions showed to be resistant to net blotch in field tests. 53 of the 76 accessions were characterized by the infection type 1-2 and leaf area damage lower than three percent using detached leaf test. In a first test 24 of the 70 tested *H. bulbosum* accessions were not infected by the virus. Two of these accessions are resistant to net blotch and to BYDV. Interspecific *H. vulgare* × *H. bulbosum* crosses are performed to transfer resistance to net blotch and BYDV into cultivated barley.

The characterization of *VRN-1* associated with heading time variation in hexaploid spring wheat germplasm from Europe and Russia

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In common wheat, *Triticum aestivum* the vernalization response and the heading time is controlled mainly by a combination of alleles at *VRN* (vernalization response) loci located on the group 5 chromosomes: *VRN-A1, VRN-B1* and *VRN-D1*. Winter wheat usually possesses recessive *VRN-1* alleles at all loci, whereas spring wheat has at least one dominant allele of these loci. Based on DNA sequence data, molecular markers were developed for each *VRN-1* allele. The *VRN-A1* alleles contain small promoter insertions, or promoter deletions, whereas the *VRN-B1* and *VRN-D1* alleles usually contain large deletions within intron 1. Recently, we identified a new *VRN-B1c* allele which has the structural peculiarities within intron 1 and affects the heading time.

Here, using molecular markers for the *VRN-1* genes we screened a set of 339 predominantly spring wheat cultivars from Europe and Russia (with adjacent regions of Ukraine and Kazakhstan) and estimated association between the presence of specific *VRN-1* haplotypes and heading time variation. *VRN-A1a*, the strongest allele conferring the spring growth habit occurs in 60% of the studied set of accessions. This allele was found in combination with the dominant *VRN-B1a* or *VRN-B1c* alleles in about 50% of spring accessions from Europe. The *VRN-B1a* allele was found in 62% accessions from Europe, whereas the *VRN-B1c* allele was found in 6.3% accessions, originating mainly from Eastern European countries. In Russia, Ukraine and Kazakhstan the *VRN-B1c* allele was detected in about half of accessions. Noteworthy, the *VRN-B1* alleles do not affect the heading time within genotypes with *VRN-A1a*. The dominant *VRN-D1a* allele rarely occurs in some South European countries (Italy, Bulgaria). The most reliable difference by the heading time was observed between the genotype containing *VRN-A1a* allele (single, or in combination with *VRN-B1a,c*) and the genotype with recessive *vrn-A1* and dominant *VRN-B1a,c*, or *VRN-D1a*. The first genotype is most characteristic for Germany, Britain, Finland, Sweden and East European countries. The second genotype is widespread in South Europe (Spain, Portugal, Italy, Bulgaria) and characterized by somewhat later heading (5-10 days) as compared to the first one.

Physical map and sequencing of a wheat chromosome arm 7DS

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Bread wheat (*Triticum aestivum*) is one of the most important crop species in the world providing a staple food for 35% of the world's population. Whole genome sequencing of bread wheat is hampered by characteristic features of the wheat genome including enormous genome size (~ 17 Gbp), polyploidy and prevalence of repetitive DNA sequences. To overcome problems due to complexity and polyploidy, a BAC-by-BAC sequencing strategy based on chromosomal physical maps has been adopted by the International Wheat Genome Sequencing Consortium.

In our project, we focus on sequencing of the short arm of chromosome 7D (7DS). A BAC library specific for the 7DS chromosome arm from a model cv. Chinese Spring (CS) was constructed, fingerprinted using SNaPshot-based HICF, and BACs were assembled into contigs using FPC software. Integration of the 7DS physical map with that of *Aegilops tauschii* (D genome ancestor) provided a framework for further merging of contigs. Reliability of the assembly was verified through LTC software. Final BAC contig assembly resulted in 931 contigs and a minimum tiling path (MTP) of 4,608 BAC clones. 52 % of the physical map has been anchored to genetic/RH maps by 630 markers allocated to 318 contigs. The BAC sequencing strategy involves sequencing 96 pools, each of 4 BACs using the Illumina HiSeq. The resulting pair-end reads are assembled using a custom assembler Sassy. This approach results in 1-7 contigs per clone. Deconvoluting the pools, scaffolding the contigs and assembly validation is performed using BAC-end and mate-pair sequences of the MTP clones and PacBio sequence data. A novel tool, BioNano restriction map, has been constructed for the 7DS arm to scaffold sequence contigs, validate the sequence assembly and size gaps. Sequences of BAC clones from the 7DS MTP will be used to anchor physical map of the 7DS. The anchored 7DS physical map, as well as the sequence of the 7DS arm, will provide an important tool for mapping and positional cloning of genes located in this part of the wheat genome.

Phenomenon of the hybrid dwarfness in wheat-rye crosses (*Triticum aestivum* L. x *Secale cereale* L.)

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Hybrid dwarfness or "grass-clumps" is a known phenomenon both in intraspecific crosses of *T. aestivum* L. and interspecific crosses between different Triticeae species carrying A, B and D genomes. Hitherto, there was no information that R genome of *Secale cereale* L. could be involved in such kind of postzygotic barriers in crosses of wheat with rye. Among the rye inbred lines from the Peterhof rye genetic stock collection of the Laboratory of Plant Genetics (St. Petersburg State University, Russia) we identified two unrelated lines 'V1' and 'V10' which produce dwarf wheat-rye hybrids in crosses with spring wheat cv. 'Chinese Spring' ('CS') and winter wheat derivative 'Priekulskaya 421' ('P421') displaying the same phenotype which can be described as *Type1-dwarfs* for wheat-wheat hybrids.

Comparative analysis of plant phenotype has shown that wheat-rye hybrids as wheat-wheat hybrids with dwarf phenotype are recognizable at very early stages of germination, have a very poor development and die within two months. Genetic analysis was performed using two interline F_1 rye hybrids ('L4'x'V1', 'L7'x'V10') as pollinators. A 1:1 segregation for the presence of normal *vs.* dwarf plants in the wheat–rye hybrids was obtained, as expected, with a total ratio of 212:196. Since 'V1' and 'V10' are unrelated by descent, allelism test was fulfilled. Because all 409 hybrid plants obtained from crosses between 'CS' and 'P421' with the 'V1 × V10' hybrid were extreme dwarfs, it was concluded, that 'V1' and 'V10' most probably carry the same gene determining hybrid dwarfness. This gene was named *Hdw* (*Hybrid dwarfism*). The allele determining the production of wheat–rye hybrids with normal development (normal or wild-type) was designated as *Hdw-R1a* and allele determining dwarfism as *Hdw-R1b*.

Histological analysis of shoot apices was performed. It was shown that wheat-rye hybrids with dwarf phenotype in ages of 21 and 45 days after germination did not further develop. Shoot apices of dwarf plants did not elongate, did not form new primordia and look as a 'dome' on the seed. This result allows to suppose that incompatible *Hdw-R1b* rye allele is involved in regulatory pathway that controls the transition from vegetative to reproductive phase in wheat-rye hybrids.

Genetic resources for the dissection of agronomic traits in European winter barley

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The characterization and exploitation of the genetic diversity of germplasm collections is a main challenge in plant genome research and plant breeding. In Italy, winter barley is predominant over spring types. For this reason we have assembled a panel of 143 winter barley cultivars representing the breeding history of this crop in Europe. The presence of both two-rowed (67) and six-rowed (76) varieties represents the main source of population sub-structure, as revealed also by Principal Coordinate Analysis (PCoA) based on SNP data, generated by means of the Illumina iSelect platform. The collection has been evaluated in comparative field trials, in order to detect significant associations between molecular and phenotypic variation, for different agronomic traits. In particular we are focusing on morphological and developmental traits that can have an important role in plant establishment and, as a consequence, on grain yield and yield components. Promising examples of Genome Wide Association Scans will be shown. A MAGIC population has been developed by inter-crossing four old and four modern six-rowed winter barley varieties, to complement the GWA studies and support the identification of agronomically superior alleles and haplotypes.

Genome wide association scan (GWAS) for seed phytic acid phosphorous, Pi and phytase activity in a collection of hexaploid wheat cultivars

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Phytic acid (PA, $InsP_6$) is the main storage form of phosphorus in cereal seeds. PA is a strong chelator of iron and other minerals, reducing their bioavailability. This phenomenon may contribute to "hidden hunger" in human populations where cereals are the primary source of nutrition. Degradation of PA in the grain to release inositol, phosphate and minerals is initiated by endogenous phytases (*myo*-inositol hexakisphosphate phosphorylase). Thus to increase the nutritional quality of wheat either reduced content of PA or increased activity of endogenous phytases in the grain is a target for molecular breeding of wheat.

In the present study we have evaluated a collection of 169 hexaploid wheat cultivars grown under ecological conditions at Agrologica, Denmark as part of the BIOBREED project[1].Content of phytic acid phosphorous (PA-P) and Pi as well as phytase activity in the grain were quantified using colorimetric assays. Significant variation was found for all three traits. Among the cultivars there were 1.4, 4.6 and 4 fold variations in content of PA-P, Pi and phytase activity respectively. Weak but significant positive correlation was observed between PA-P and Pi, while none of the three traits showed significant correlation to grain weight.

The wheat population were genotyped with 2500 DArT (Diversity Arrays Technology) markers distributed over the wheat genome to identify markers associated with the three traits. GWAS was carried out using compressed mixed linear models accounting for familial relatedness in the population. Using this approach, potential QTLs were identified for the three traits, information that may in the future lead to development of markers useful for breeding of improved cultivars.

SNP genotyping of wheat accessions from Kazakhstan

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Wheat is the major crop in Kazakhstan growing over 13 million hectares annually. Due to harsh environmental conditions the average spring wheat yield in Kazakhstan is less than 1.3 t/ha. One of the ways to improve the production of wheat is to employ new technologies to discover new stress resistant genes. Ninety (90) spring wheat cultivars officially released in Kazakhstan were genotyped by 90K SNPs of Illumina assay by using *TraitGenetics GmbH* (Germany) facilities. The quality of screening of wheat accessions by 90K SNP array was high and yielded nearly 62K SNP markers. 15K polymorphic SNPs with less than 10% of missing data were used for further phylogenetic analysis. The Neighbor joining phylogenetic tree suggested separation of all accessions to four clusters with 3rd divided to two sub-clusters and 4th cluster to 6 sub-clusters. Genetic variation of the collection from Kazakhstan was compared to samples from Asia, Australia, Europe and North America. The analysis is allowed to identify a number of unique and rare alleles found in collection from Kazakhstan. The data used for QTL mapping of yield components based on association mapping method.

The work was done as a part of international ADAPTAWHEAT project of 7th Framework Program of the European Union (the coordinator of the project is Dr. S. Griffiths, John Innes Centre, Norwich, UK). The authors acknowledge the work done by 90K SNPs Illumina assay developers.

Comparative genetic and phenotypic variation of USA and Kazakhstan barley accessions

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The barley cooperative project in Kazakhstan has started in 2009 and consisted from the participants of 8 different local research organizations. The main goal of the project is coordinated barley genome research for breeding of high yield malt and feed barley of two-rowed and six-rowed plants. The material consists from 120 perspective lines and commercial cultivars of Kazakhstan and 580 accessions of the USA Barley CAP received from Dr. T. Blake (Montana State University, USA). The USA accessions were previously genotyped (3,072 SNPs) by USA Barley CAP. The accessions from Kazakhstan were genotyped by selected 384 SNP markers by using facilities of Okayama University, Japan. On the first stage of field trials the research material was grown three years in 7 different stations (two randomised repetitions). On the second stage selected 96 accessions were tested on 10 meter blocks of experimental plots in five major barley growing regions in 2012-2013. The association mapping approach for the major agronomic and grain quality traits was tested by using MLM method of TASSEL. The comparative assessment of accessions has allowed identifying prospective lines for major barley growing regions of Kazakhstan.

Genome wide associations study (GWAS) of juvenile barley (*Hordeum vulgare* L.) genotypes for drought stress induced leaf senescence and drought stress tolerance

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Premature leaf senescence induced by external stress conditions, e.g. drought stress, is an important factor for yield losses. Research in drought stress tolerance has become more important in agriculture worldwide as due to climate change the number of drought periods will increase in the future. Therefore, tolerance to drought stress has become an important goal in barley breeding. The aim of this project is to identify genomic regions involved in drought stress induced leaf senescence and drought stress tolerance in early developmental stages of barley (*Hordeum vulgare* L.) by applying genome wide association studies (GWAS).

In greenhouse pot experiments 156 barley genotypes including 113 German cultivars and 43 accessions of the Spanish Barley Core Collection (SBCC) are tested for their response to early drought stress and induction of leaf senescence under control (70% of maximal soil water capacity) and stress conditions (20% of maximal soil water capacity). At the end of a four weeks stress period chlorophyll content and chlorophyll fluorescence which are indicators of leaf senescence, as well as the content of free proline, total content of soluble sugars, osmotic adjustment and the aboveground biomass production indicative for drought stress were determined. The experiments of preliminary two year´s trials revealed variability in the parameters representing different and specific adaption mechanisms to drought stress.

In parallel this set of genotypes was analysed with the 9k iSelect SNP-chip available for barley. In summary 6807 SNPs turned out to be polymorphic. Those showing a minor allele frequency >5% and being mapped were used for GWAS taking into account population structure and kinship. Based on this procedure, significant marker trait associations for the above mentioned traits were detected on all barley chromosomes and NCBI Blast search revealed that respective SNPs are in some cases located in genes related to drought stress.

Ways to unlock barley´s secondary gene pool for breeding with Next-Generation-Sequencing

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Crop wild relatives (CWR) have been recognized as a source of beneficial traits to a given crop species and to overcome the erosion of genetic diversity resulting from domestication and small effective population size in elite breeding programs. The efficient utilization of crop-wild introgression lines for improving elite germplasm has largely been hampered due to the lack of suitable molecular tools for locating introgressed segments and decreasing their size during subsequent crosses. Marker development in CWR is mainly restricted by limited access to the genomic information of these species. We explored the usefulness of Next-Generation-Sequencing technology for developing and scoring molecular markers in a diploid introgression line of cultivated barley (*Hordeum vulgare* L.) containing chromatin from its wild relative *Hordeum bulbosum* L. We used a recently developed whole exome capture assay in combination with a custom SNP genotyping assay as well as two-enzyme genotyping-by-sequencing to allocate the introgression interval and to genotype progeny segregating for the introgression. Both methods provided fast and reliable detection and mapping of the introgressed segment and enabled the identification of recombinant plants avoiding tedious and iterative steps of marker development. This will ultimately pave the way for the detailed characterization of *H. vulgare/H. bulbosum* introgression lines.

Effects of the tiller inhibition (*tin*) gene in winter wheat

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Wheat (*Triticum aestivum* L.) with the 'Gigas' or 'Big Ear' characteristics are easy to distinguish from other commercial wheat. The main distinguishing characteristics for 'Gigas' wheat include: uniculm plants, large number of spikelets per spike and thick stem. The reduction in tillering is due to a single recessive gene, designated *tin*, for tiller inhibition. The *tin* gene located on wheat chromosome 1AS. It has been reported that spring wheat lines containing the *tin* gene had a 90% reduction in tillering when planted during the spring and summer, but if planted during late fall/winter the effect was only 30-50% reduction in tillering.

In order to investigate the effect of the *tin* gene in a winter wheat background two sets of crosses were made. The first being a three-way cross between a CIMMYT line containing the *tin* gene, a large spike adapted winter wheat line from Purdue, and a profuse-tillering line. Our goal was to produce a population that exhibited a wide range of traits for tillering, spike size, stem diameter, etc. The second cross was between a landrace from Afghanistan that produces a large ear and another large spike adapted winter wheat line from Purdue. From this cross, our goal was to look at another source for the large spike characteristics to be used for breeding purposes.

Our preliminary observations indicate that in winter wheat genetic backgrounds the reduction in tillering effect of the *tin* gene is not as significant as in spring wheat; while the spike size, kernels/spikelet and stem diameter are increased, as seen in spring wheat. Our hypothesis is that in winter wheat, we can achieve moderate increase in spike size, kernels/spike, kernel weight and stem diameter; with only a moderate reduction in tillering. Through plant breeding techniques, our goal is to combine these traits that provide underutilized variation with current high yielding components to improve yield potential.

Development of bioinformatics workbench, bex-db, available for comprehensive analysis of barley expressed genes

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Despite its large and complex genome, a physical, genetic and functional sequence assembly of the (*Hordeum vulgare*) genome was reported by IBSC, providing a platform for future comparative and functional analysis in this important crop (The International Barley Genome Sequencing Consortium, 2012). Here we present the Barley Gene Expression Database (bex-db), including both sequences and expression profiles of the barley genes, as a useful resource for further genomics studies and development of genome-based tools to enhance the genetic improvement progress of cereal crops as well (Tanaka, *et al*., 2013). Besides 29,782 completed full-length cDNA (FLcDNA) sequences, our bex-db also contains more than 309,000 partial and novel expressed sequence tags (ESTs). These EST sequences constitute 22,148 sequence clusters and 3,380 singletons. Moreover, the bex-db provides expression profiles for up to 36,632 barley genes obtained from the microarray analysis under desiccation, salt, and cold stresses and ABA treatment (Matsumoto *et al*., 2014, in press). Users can access the above data easily via any of the following search options; keywords, sequence homology and gene expression profiles. A genome browser was also developed within our bex-db to display the chromosomal locations of barley FLcDNAs and ESTs, wheat (*Triticum aestivum*) transcripts as well as gene models of the two wild diploid grasses (*Aegilops tauschii* and *Triticum urartu*) on the IBSC barley genome sequence, that should be largely useful not only for visualizing the genomic structures of barley genes but also understanding the sequence divergence of orthologous genes among the whole Triticeae species. To be user-friendly, a function of internal links crossing the different datasets within the bex-db was added. This work was supported by grants (TRG1008, GIR1001 and TRS1001) from the MAFF.

URL for bex-db: http://barleyflc.dna.affrc.go.jp/bexdb/index.html

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The species diversity of feed cereals - wheatgrass in Kazakhstan

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Kazakhstan has great potential for the successful development of animal husbandry. The pastures occupy 187 million hectares of land, which is 70 % of the country. Potential of forage of the territory - 24 -30 million tons of feed units produced annually (Ismailov, B., et al, 2008).

In the structure of the share of fodder cereals account for about half of all of feeds derived from agrocenosis as well as with the natural meadows and pastures. Family of grass - Poaceae in the republic represented 482 species belonging to 101 genus. Widespread representatives of the genera *Stipa, Festuca, Agropyron, Elytrigia, Dactylis, Bromus, Phleum, Phalaris* and others.

Genus wheatgrass – *Agropyron* is of great importance as a valuable feed plants adapted to soil and climatic conditions of arid regions. They are drought resistant, grow early spring and late autumn to vegetate, tolerate grazing animals. The most common in Kazakhstan are the following: wheatgrass of comb - *Agropyron cristatum*; narrow ears of wheatgrass (Siberian) - *A. fagile*; wheatgrass of desert - *A. desertorum*; wheatgrass of crested - *A. pestinatum*; wheatgrass of imbricate - *A. imbricatum.*

Plant genetic resources are a major factor in sustainable agricultural production - are the biological basis of food security and livelihood of any country. Delivering exceptional value to biodiversity problem, Kazakhstan joined the "Convention on Biological Diversity". Gene pool of crops in Kazakhstan is more than 79.0 thousand samples. In this the proportion of forage crops is 12.8 thousand samples. For the formation, store, replenishment, explore and create ex situ collections of cultivated and wild flora, in order to further their use in breeding gene pool was created wheatgrass in 3154 volume of the sample following species identification: *Agropyron cristatum* (Crested wheatgrass) - 880*; A. desertorum* (F. desert) - 605; *A. fragile* (wheatgrass sibirsky) - 324; *A. pestinatum* (wheatgrass grebnevidny) - 816; *A. imbricatum* (wheatgrass cherepitchaty) - 329.

Diversity of crop wild relatives to improve the breeding of grain and forage crops in Kazakhstan

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Collections of Kazakhstan Republic contain more than 50, 0 thousand samples of agricultural cultures. However, the wild relatives of agriculture plants of local flora are submitted in collections of small volume. The wild relatives are insufficiently investigated from the point of a genetic variety and breeding utility. The wild relatives are capable to decide problems of resistance to diseases, wreckers, cold, drought and capable to expand limited genetic base of the modern varieties. Natural ecosystems of Kazakhstan hold the important genetic resources for a feed and agriculture: more than 120 species from 6000 of Kazakhstan flora are wild relatives of agricultural plants (N.V.Pavlov, 1956). The preservation unique genetic variety of plants very important from the point of natural erosion caused industrialization and changes in agriculture.

Many wild relatives of grain and fodder cultures are under direct threat of genetic erosion. In Kazakhstan till now are used as fodder cultures wild relatives of *Triticum L*, *Hordeum L, Avena L,* that can result in their complete destruction. As a result of expeditions conducted last 15 years the populations of grain and fodder cultures wild relatives from various landscape zones (steppe ecosystems, large mountain systems: Northern Tyn –Shan (Zailiysky Alatau, Dgungarsky Alatau), Southern Altai and Western Tarbagatay) have been surveyed. The seeds of 28 species from 13 genus wild relatives of *Poacea* family are assembled: 1. *Aegilops L*.; 2. *Bromopsis Fourr*.; 3. *Elytrigia Desv*.; 4. *Hordeum L*.; 5. *Agropyron Gaertn*.; 6. *Helictotrichon Bess.*; 7. *Leymus Hochst*; 8. *Elymus L*.; 9. *Panicum L*.; 10. *Koeleria Pers*.; 11. *Melica L*.; 13. *Psathyrostachys Nevski.* The samples and data connected to them will be used in the national breeding programs of grain and fodder cultures improvement.

Effect of gamma-radiation on ISSR and AFLP profiles of amaranth when comparing to biochemical analyses

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M_8 generation of amaranth plants positively selected for weight of thousand seeds- five mutant lines C15, C26, C27, C82 of genotype Ficha (*Amaranthus cruentus* L.) and three D54, D279, D282 of hybrid K-433 and two control genotypes were used to analyze length polymorphism. AFLP and ISSR analyses were used to determine which of several gamma-irradiated mutants of amaranth Ficha cultivar and K-433 hybrid are most genetically dissimilar to their non-irradiated control genotypes. AFLP analyses were done according to the AFLP Plant Mapping Protocol and fragments were separated by capillary electrophoresis. ISSR reactions were done with 1 U Taq polymerase (Applichem) and 600-800 nmol × dm-3 primer. Amplifications were carried out with the program: 95°C for 4 min followed by 45 cycles at 95°C for 60 s, 52°C for 60 s, and 72°C for 2 min and a final cycle at 72°C for 10 min. AFLP and ISSR electrophoreograms were evaluated and binary matrix were constructed on the base of the peaks presence or absence, Jaccard's coefficients were calculated and cluster analysis UPGMA was conducted. The profiles of summary dendogram for ISSR and AFLP markers of the Ficha cultivar mutant lines were more similar to the Ficha control than profiles of K-433 hybrid mutant lines. Based on ISSR, mutant line C82 share the specific position to the Ficha control genotype and the result corresponds to the results of the biochemical analyses of the same mutant lines as in this study, where C82 line is statistically different as the rest of the analysed Ficha lines. In AFLP, mutant lines C15 and C27 positions correspond to the results of the biochemical analyses, where both of them are reported as statistically different to the Ficha in the content of prolamins plus glutenins and C26 is reported as to having the lowest content of total proteins when compared to the Ficha.

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Session II

Grain Yield

Evaluation of water use efficiency, biomass production and selected root traits of wheat (*Triticum aestivum* L.) under drought conditions

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Water use efficiency (WUE) is an important physiological trait for assessment of biomass production in drought conditions. The present research was designed to investigate the relationship of WUE, biomass production and selected root traits using hundred wheat genotypes cultivated in Pakistan in replication of three. The result showed that WUE is positively correlated (r= 0.982) to biomass production and negatively correlated to selected root traits (No. of saminal root (NSR) r = -.030, root diameter (RD) r = -.030 and Maximum root length (MRL) r = -.068). In drought conditions thirteen out of hundred genotypes viz. Kiran (2.155 g/Kg), Janbaz (1.634 g/Kg), ZA-77 (1.620 g/Kg), AS-2002 (1.612 g/Kg), Dirk (1.593 g/Kg), Zamindar-80 (1.573 g/Kg), Lasani-08 (1.569 g/Kg), Mehran-89 (1.569 g/Kg), Pirsabak-85 (1.561 g/Kg), Iqbal-2000 (1.553 g/Kg), Punjab-76 (1.549 g/Kg), Barani-70 (1.544 g/Kg) and Bakhtawar-94 (1.505 g/Kg) were showed the highest WUE while in Sonalika lowest WUE (0.376g/ Kg) and biomass production (0.197/g) was recorded. The present study reveals that it is needed to improve the root system function rather than a strong root growth for wheat high WUE and biomass production in drought conditions.

To study the effectiveness of various bio-pesticides to manage the *Aphis* spp. (Hemiptera: Aphididae) on the wheat (*Triticum aestivum* L.) crop in Punjab, Pakistan

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The wheat is an important cereal crop, grown as a food grain in Pakistan and worldwide. The experiment was conducted on wheat variety, Pasban 90, at Post Graduate Agricultural Research Station (PARS), University of the Agriculture Faisalabad during 2009-10 to compare the effectiveness of a bio-pesticide against the wheat aphid. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The first time population was observed from 10 February 2012. The highest population was recorded at second last week of the March. The data of the aphid were recorded from 130 tillers of the wheat, 72, 168, 336 and 512 hours after the application of the bio-pesticide from each treatment. The five bio-pesticides have been used. The neem leaves extract showed the maximum control after the 512 hours (4.12 aphid / tiller) of application. The release of *Chrysoperla Carnea* also gave a significant control. The main objective of the study is to control the wheat aphid by using bio-pesticide and ultimately to increase the high yield potential of wheat crop. The overall results were equally beneficial to researchers, scientists and farmers community to protect the wheat crop and to increase the yield production of crop plants.

Participatory varietal selection of malt barley varieties for yield and related traits at Gusha Shinkurta, West Gojam, Ethiopia

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Participatory variety selection was conducted to select alternative genotypes of malt barley with better yield, quality and related traits through farmers' evaluation and selection. The study investigated the application of participatory varietal selection to identify malt barley cultivars that are suitable for the malting and brewing industry.Ten released and promising genotypes were evaluated at Gusha in western Gojjam, North West Ethiopia. Two types of trials were designed, grandmother trial consisting of 10 genotypes in RCBD design with three replications at one farmer site and three mother trials with one replication each at three farmer's fields in the 2010 cropping season. Based on farmers selection criteria genotypes were scored using matrix ranking method. Yield and some related characters were estimated. Significant variation was observed among genotypes evaluated for important quantitative traits in both trials indicating presence of variability. The mean yield ranged from 1.0 to 2.4 ton per hectare. The highest yielding genotype was HB1533 (2.4 ton/ha) followed by EH 1877/F4 (2 ton/ha) and IBON 174/03 (1.9 ton/ha). Poor yield was recorded for Beka (1 ton/ha) and it was susceptible to scald and net blotch. Cultivar HB1533 had the highest rank followed by HB120 and IBON174/03 in the grandmother trial. In the mother trial, HB52, HB120 and HB1533 were ranked first, second and third, respectively. The variation of the two trials result were due to that evaluation was done by different groups and they had different preferences.

Integrated strategies to control *Fusarium* head blight and deoxynivalenol contamination in winter wheat subjected to different soil tillages

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Fusarium head blight (FHB) disease and deoxynivalenol (DON) contamination of wheat grains depend on climatic conditions, but also on agronomic factors. The primary reservoir of *Fusarium* inoculum is debris from the previous crop: the disease is more severe if the preceding crop is maize. Moreover, it is clearly reported that no-tillage increases the frequency of FHB compared to ploughing. The objective of this study was to investigate the effect of the application to crop residues of microorganisms with an antagonist action, in order to reduce the risk of *Fusarium* dispersal with conservative tillage practices.

Four field experiments have been set up over two growing seasons in Northern Italy to evaluate the effect of previous crop residue management through tillage (direct sowing, minimum tillage and ploughing), the application of *Trichoderma asperellum + gamsii* before tillage and the fungicide application (prothioconazole + tebuconazole) at heading on common wheat following maize for grain, according to a full factorial scheme, with 4 replications.

As expected, FHB severity and DON contamination significantly increased with the density of the residues left in the first 10 cm of soil; DON content was not significantly different between minimum tillage and direct sowing conditions. The application of *Trichoderma* spores before tillage resulted in a clear reduction (5-20 times) of *Fusaria* infection on soil and debris collected at wheat heading. However, in all the tillage practices the FHB symptoms and the DON occurrence were not significantly affected by this antagonist action. On the other hand, the fungicide application significantly reduced DON content by 66%, 69% and 59%, in ploughed, minimum tillage and direct sowed plots, respectively.

This research underlines that integrated multiple strategies are the effective management means of reducing DON contamination in wheat. The use of antagonist action of microorganisms on previous crop residues need to be better investigated, in order to quantify their effect to prevent DON content in wheat grain.

The research was sponsored by the Italian Ministry of Agriculture in the framework of the Project MICOPRINCEM.

Soil and foliar late-season nitrogen applications for enhanced protein content and quality in improver common and durum wheat

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High grain protein content (GPC) is a desired trait for improver common wheat and durum wheat. Nitrogen (N) fertilization, in particular the late-season application , is the main factor affecting storage proteins as well as technological quality. The aim of this study was to compare, in different soils and growing seasons, the effect of late-season N fertilization strategies, through granular top-dressed soil or foliar applications on wheat quality.

Field experiments were set up over 2 growing seasons in 2 different sites, sandy-loam and silt-sandy-loam, in North West Italy. In each site the effect of N fertilization was evaluated on 2 common and 2 durum wheat cultivars, following a full factorial scheme with 4 replications. Late-season N was soil-applied (40 kg N ha^{-1}) as ammonium nitrate at heading or foliar sprayed (5 kg N ha^{-1}) at flowering and compared to a control without N fertilization after the vegetative growth stages. From tillering to stem elongation a total of 130 kg N ha^{-1} was applied to all treatments as ammonium nitrate. The following parameters were recorder for both crops: flag leaf greenness, grain yield, test weight, thousand kernel weight (TKW), grain hardness and GPC. Alveographic and farinografic analyses were performed for common wheat, while the gluten index and the yellow berry incidence were recorded for durum wheat.

In all the experiments, grain yield and TKW were not affected by late-season N fertilization, while soil application at heading let to a significant increase of flag leaf greenness (+12%) and GPC (+13%) for both crops. Moreover, this fertilization strategy led to higher test weight (+1.4%), kernel hardness (+14%), W (+42%) and lower P/L (-18%) for common wheat and lower yellowberry percentage for durum wheat (-29%).

N foliar fertilization at anthesis resulted in a general lower improvement of GPC and quality parameters. Moreover, this strategy was more effective to enhance GPC in the soil with finer texture and higher cation-exchange capacity, suggesting that foliar fertilization, more than a direct effect on plant N nutrition, could play an indirect effect to maintain a higher post-anthesis N uptake, through a delay of leaf senescence.

The research was sponsored by the Piedmont Region (Project QUALICHAIN).

Identification of QTLs for agronomic traits introgressed in an elite cultivar from a promising barley landrace

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Crop landraces are of considerable importance as genetic resources, especially in breeding for biotic and abiotic stress. A group of Spanish barley landraces have shown to yield more than cultivars at low production sites. The best of these lines are being introduced through backcrosses in the national barley breeding program. The objective of this study is to identify favourable QTLs for interesting agronomic traits contributed by landrace line SBCC073, which might be useful when they are introgressed in elite cultivars. A population of 100 BC1F5 lines was derived from the cross between an elite cultivar (Orria) with high productivity and Spanish landrace (SBCC073) with high yielding at low production. A genetic linkage map was developed for this population using 1227 SNPs of good quality genotyped with a DArTseq® genotyping-by-sequencing assay. The population of 100 BC1F5 lines was evaluated in the field in Zaragoza (Spain) during two years (2011, 2013) for grain yield (2011, 2013), flowering time and plant height (2013). A genetic map was constructed with programs Joinmap® and MSTMap. The genetic map resulted in 11 linkage groups, covering a total distance of 983 cM, with 4 complete chromosomes (1H, 3H, 4H and 5H) and three fragmented ones (2H, 6H and 7H). Three QTLs for grain for grain yield were detected in 2013, but for only one of them, on 5H, the yield increasing allele was contributed by SBCC073, whereas Orria alleles increased yield at QTL on 3H and 6H. Only one flowering time QTL was found, on chromosome 3H.

Evaluation of heterosis in Australian wheat

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Yield improvements from conventional crop breeding in wheat have tapered off over the past decade. Consequently, there is renewed interest in technologies that offer major yield advantages particularly for low yielding environments. One of the most promising options is to capture the yield benefits from heterosis in a hybrid wheat program. The impact of heterosis in wheat has not received the level of attention seen in other crops, particularly maize. Although wheat is a self-pollinated species, reports of the yield benefit in hybrid range from 5% to over 20%, depending on the study and the nature of the crosses. In order to evaluate the impact of heterosis in Australian elite germplasm, crosses were made between three Australian varieties and ten diverse lines from different regions around the world. The diverse lines were chosen based on genetic diversity detected with the 9K SNP array as well as the country of origin. Thirty hybrids and their parents were evaluated under controlled conditions in The Plant Accelerator- Adelaide where automated images were taken every day from 20 to 60 days after sowing. Growth rates of all genotypes were obtained from the data generated through the Lemnatec automated imaging system. Twenty-six of the hybrids and their parents were also evaluated in semi-controlled field conditions at Urrbrae (South Australia) under well-watered and drought conditions. Flowering time, plant height, grain number per plant and grain weight per plant were recorded in both experiments. The field experiments indicate that some hybrids show mid-parent and better-parent heterosis for several traits providing a platform for the evaluation of heterosis. Data obtained from the image analysis and field experiments will be compared to evaluate the effectiveness of automated high-throughput phenotyping to predict field performance.

Selection QTL in barley breeding lines detected by combining genotyping-by-sequencing with reference genome information

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This study is a retrospective analysis of an elite cross from the Spanish National Barley Breeding Program. The cross was one of the most successful crosses produced in the breeding program in the past 20 years. The progeny from this cross has been investigated at two points in the program, before (F2) and after (F8) undergoing selection, through the analysis of allelic frequencies at a number of genetic loci with molecular markers, to identify genomic regions with selection footprints due to the breeding process. The crosses were sampled at two generations, before selection (F2) and after 6 cycles of selection (F8). The F2 plants were genotyped with microsatellites, whereas 31 F8 lines were surveyed for SNPs and PAVs using a genotyping-by-sequencing (GBS) system, DArTseq. The GBS markers were aligned to the barley physical maps and, after curation, over 3000 markers were still available for the analysis. We found 14 regions in the F8 lines with allele frequencies beyond the thresholds (FDR<0.05), indicating selection, 11 towards parent Orria and 3 towards Plaisant. These selection QTL co-located just partially with QTLs detected though classical linkage mapping in a RIL population of the same cross. These selection QTL could be directly useful for barley breeding, either through marker assisted selection or genomic selection.

Characterization of nitrogen use efficiency in a winter wheat collection

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Winter wheat is among the main cereals that has the greatest importance in human nutrition. It is well known that yield and quality of winter wheat could be dramatically increased through the application of N fertilizers. However, it has been also proved that only a low rate of the applied fertilizers can be utilized by the crops. Therefore, the identification of varieties with better nitrogen (N) uptake and N utilization efficiency is a very important topic. Varieties with better nitrogen use efficiency requires less nitrogen fertilizers, that makes the cultivation more economical and environmentally friendly. The main objective of this study is to investigate and characterize the N-use efficiency in a winter wheat collection.

Ninety-six winter wheat lines were investigated under field condition in 2013. Two different nitrogen fertilization levels were applied, an extensive and a favourable N level was adjusted. The response was characterized by the measurements of agronomically important characters (grain yield, spike number, thousand grain weight, plant height, heading time, biomass and harvest-index) and physiological parameters like relative chlorophyll concentration. The main characteristic parameters for the nitrogen usage, the N-use efficiency (NUE) and its two components, namely nitrogen uptake efficiency and nitrogen utilization efficiency were also measured and calculated.

The first-year dataset of the three year experiment has been analysed. To find out, which genotype is recommended for intensive and which one is for extensive cultivation, the performances of the varieties were characterized under low and also under favourable nitrogen supply. The varieties were also ranked based on the increased level of their performance due to the nitrogen supply. Correlations between the measured phenotypic data and the NUE parameters were also calculated. This was the first dataset to be analysed, and the same set of winter wheat lines will be screened for two further years.

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Transgenic winter wheat expressing a barley sucrose transporter exhibits increased grain yield and accelerated plant development

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Transgenic winter wheat lines (HOSUT) expressing the barley sucrose transporter HvSUT1 (SUT) (Weschke *et al*. 2000) under control of the hordein B1 promoter (HO) of barley exhibit increased sucrose uptake capacity of the developing grains (Weichert *et al*. 2010). Three transgenic HOSUT lines harbouring a single homozygous copy of the transgene at independent genomic loci were developed by *Agrobacterium*-mediated transformation of the winter wheat variety Certo. The transgenic lines are free of any marker gene.

The HOSUT lines were evaluated during three consecutive growing periods under field-like conditions and show increased grain yield, increased number of spikes per plot and a significant gain in thousand grain weight. Higher thousand grain weight is the most consistent yield-related parameter of the HOSUT lines (Saalbach *et al.* 2014) shown along with increased grain yield also under depleted N-fertilization when compared with the isogenic cv. Certo.

More detailed morphological analyses revealed that HOSUT grains have enhanced length, width and area. The differences between HOSUT and Certo are higher in grain width than length, indicating that the transgene effects are mostly evident during early development when the caryopses grow under filial control (Saalbach *et al.* 2014). In-depth molecular and biochemical analysis showed an altered biochemical profile of mature HOSUT grains in comparison with those of Certo. Higher iron and zinc concentrations (20-40%) were detected along with similar or slightly decreased relative protein content and more clearly decreased sucrose content. The latter might indicate that HOSUT grains develop under sucrose depletion.

Additionally to the grain effects, HOSUT lines show accelerated seedling and plant development resulting in earlier flowering and ripening when compared with Certo.

Early developmental traits of wheat as affected by different photoperiod insensitivity alleles

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Photoperiod genes are important in wheat breeding to fine-tuning flowering time, which involves three phases: vegetative (when leaf primordia are generated), early reproductive (when spikelet primordia are formed) and late reproductive (initiation of florets within spikelets). The effects of photoperiod genes on flowering time are well known, but to what degree they affect the duration of these three phases has been scarcely studied. It is during these phases when source- and sink-organs are being formed, and then quantifying the effects of *Ppd* alleles on these particular phases may be relevant. The objective of this study was to evaluate the impact of photoperiod genes on (i) duration of these phases, (ii) initiation of leaves and spikelets, and (iii) dynamics of appearance of leaves and tillers (besides the coordination between them). A field experiment was conducted during 2012 in Bell-lloc (NE Spain) using NILs kindly provided by S. Griffiths (John Innes Centre). These lines have photoperiod insensitive alleles introgressed in the A, B and D genomes (*Ppd-A1a*, *Ppd-B1a*, *Ppd-D1a*, respectively) from the photoperiod sensitive variety Paragon, which acted as the recurrent parent. Photoperiod insensitivity alleles accelerated flowering time with respect to Paragon. The magnitudes of the acceleration depended upon the both the specific allele and its source. Introgression of *Ppd* alleles reduced the length of the vegetative phase decreasing final leaf number (FLN) and that of the late reproductive phase due to both a lower FLN and a shorter phyllochron. The early reproductive phase was not always modified; however, spikelet number was decreased. The advancement of the development by these alleles resulted in a lower maximum number of tillers though there was no effect on the number of fertile tillers. The magnitude of these effects varied with the allele and the source. Photoperiod alleles had distinct effects on the duration of the different phases and could be potentially used to produce well adapted varieties with a tailored partitioning of developmental time between different phases.

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Dynamics of floret initiation/degeneration in wheat NILs for relevant photoperiod genes

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World population growth demands an increase in productivity. Due to spatial restrictions, genetic improvement will need to play a major role. Wheat yield is linearly related to grain number per m^2. As wheat is a cleistogamous plant, understanding the physiology of floret development and ascertaining the role that particular alleles might play to determine the final number of fertile florets may be useful to achieve grain number and yield.

Photoperiod genes have been shown to affect the duration of all pre-flowering phases in wheat including that when florets develop. We aimed to evaluate changes in the dynamics floret development among different wheat NILs for Ppd genes and to integrate these developmental patterns in the dynamics of floret initiation/death establishing (i) the relevance of this rate in the genotypic differences in fertile florets and grains and (ii) the effects of particular Ppd alleles (and their sources) on these dynamics and on setting a particular level of spike fertility. For this purpose, a field experiment was carried out in Bell-lloc (Lleida, NE Spain) in which we grew 34 wheat NILs differing in Ppd alleles produced by the John Innes Centre. Treatments were arranged in a completely randomized design with three replications. From the onset of stem elongation onwards, plants were sampled frequently, the spikes dissected and then within central spikelets floret primordia were counted and their stages of development determined. At anthesis and maturity time, a sample of each plot was harvested and the number of fertile florets and grains was counted, respectively.

In all NILs carrying the photoperiod insensitivity allele the time for floret development was reduced causing a decrease in the total number of fertile florets per spike at anthesis and affecting the spike fertility. Consequently the number of grains per spike at maturity was also reduced in most of the NILs. Although this pattern was generalised across the different NILs, the magnitude of the effects showed variation among the different sensitivity genes.

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Environmental effects on the estimation of yield genetic gains of Mediterranean durum wheat

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Estimations of the genetic gains for wheat yield based on field experiments may differ between environments due to genotype x environment interactions. Here we compared the estimations of genetic gains for yield and yield components in two Spanish latitudes (North, 41°40'N, 0°20'E and South, 37°10'N, 3°35'E), and identified the environmental covariables responsible for the differences observed. Six field experiments were conducted during three growing seasons (2003-2005). The northern site is characterized by low temperatures during winter and spring and the southern one by mild winters and high temperatures after anthesis. Yield and yield components (number of spikes m^{-2}, number of grains per spike and thousand kernel weight) were determined in a historical series of 12 Italian and 12 Spanish durum wheat cultivars released in different periods during the 20^{th} century. The cultivars of these two countries showed similar trends. Estimated yield gains were larger in the South (71% or, in relative terms 0.74% y^{-1} from old to modern cultivars) than in the North (23.7% or 0.30% y^{-1}); however, differences between relative genetic gains (RGG) for yield were not associated with the yield potential of the environment. In both latitudes, the number of grains per spike and the number of spikes per m^2 explained the largest proportion of yield improvement variation, but for both traits the RGG was larger in the South. Small reductions in kernel weight over time were recorded in the North, while no significant changes were detected in the South. Relative genetic gain for yield was significantly and positively related to thermal time and average daily maximum temperature from sowing to anthesis. Our results suggest that the higher temperatures recorded until anthesis in the South had a more negative effect on the yield of old cultivars than of the modern ones. Thus, yield differences between old and modern cultivars were larger in the South than in the North, resulting in greater estimated genetic gains under Southern conditions.

Heterosis exploitation in the genus *Triticum* L. using intra- and inter-specific crosses

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The growth of the human population and climatic changes has an important impact on the activities related to production and cultivation of wheat (*Triticum* spp.). Considering that within the last twenty years area intended for wheat production have been constantly shrinking on a global level and the global wheat consumption trend indicates a steady upward direction, the only solution against the shortage of this cereal species seems to be a faster yield growth on a local, regional, national and global level. In the Central Europe, the annual contribution of breeding work to the growth of an average grain yield of common wheat (*Triticum aestivum* L.) is practically not evident. This means that in the near future it will be necessary to find alternatives for inbred varieties which are prevailing in the wheat production. According to several authors, the alternative to production of inbred varieties lies in the exploitation of heterosis using F1 hybrids, and in addition intra- and inter-specific crosses.

During the 2011/12 season 43 F1 intraspecific hybrids were analyzed and compared to 42 F1 hybrids (*T. aestivum* × *T. aestivum*) for grain yield heterosis. Based on the results we could conclude that the creation of hybrids from crosses between wheat and their relatives having a similar genome (eg. *T. aestivum* L. × *T. spelta* L., *T. aestivum* L. × *T. compactum* HOST, *T. aestivum* L. × *T. sphaerococcum* PERCIV., *T. aestivum* L. × *T. macha* ...) may result in high heterosis for grain yield (MPH > 60 %, BPH > 10%). In addition we will verify the potential of interspecific (synthetic) hybrids for grain yield during the 2013/14 season. More than 50 synthetics based on ABD genome (synthetic hexaploid wheat) will be evaluated and compared to the elite wheat genotypes for parameters associated with the grain yield (eg, number of shoots , spike length, ...). The results obtained will be an important contribution to efforts of heterosis exploitation in the region.

Effect of temperature on *HvCEN-EPS2* and *HvBM5A_Vrn-H1* by environment interaction in multi-environment barley trials across the Mediterranean Basin

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Abiotic stress such as severe drought events have large influence in grain yield in the rainfed region of Mediterranean basin. Environmental factors such as precipitation have effects on growth, development, biomass and grain yield. Factorial Regression has been used in recent years to describe differential genotypic sensivities to environmental co-variables. We checked the sensibility to environmental factors on QTLs effects for flowering time and yield in the biparental population 'Nure' x 'Tremois', in 18 field trials across the Mediterranean Basin during two consecutive years (2004, 2005). By regressing QTLs effects and environmental covariables, we found two QTLs for both Days to Heading (DtH) and Grain Yield (GY) that are strictly related with temperature and temperature based variables during the growing cycle of the crop. These two QTL seems to correspond to two well known development genes the earliness per se or early maturity locus HvCEN_EPS2 and a vernalization gene, HvBM5A_Vrn-H1. Factorial regression for DtH showed a non cross-over QTL.E interaction for HvCEN_EPS2 that results in difference of few days in flowering time due to the 'Nure' allele that promote early flowering. On the other hand HvBM5A_Vrn-H1 show a crossover interaction, in this case the spring allele from 'Tremois' seemed to accelerate heading date in mayor part of field trials, when subjected to high temperatures during vegetative phase. In three trials, characterized by lowest temperatures and for the highest number of days with temperature under 0º C, the recessive winter allele vrn-h1 from 'Nure' seems to be favorable by hastening flowering time. Factorial regression for GY showed a quantitative QTL.E interaction for HvCEN_EPS2. In this case the allele from 'Nure' seems to increase GY due to early flowering mediated by temperatures. The HvBM5A_Vrn-H1 allele from 'Nure' seemed to increase GY in trials with lowest temperature during vegetative phase, due his effects on vernalization under short day. On the other hand, in two field where sowing was performed late, and temperature during the vegetative step were higher, we found significant positive effect on GY associated to the HvBM5A_Vrn-H1 allele from 'Tremois' that is a spring modern high yield cultivar.

Genome-wide association studies for yield-related traits in a diverse collection of 2-rowed barley landraces adapted to a wide range of temperate climatic conditions

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Barley landraces are populations of genotypes that evolved under human selection in subsistence agriculture. Since the beginning of the 20th century, these populations have been gradually replaced by modern cultivars, which are higher yielding under optimal conditions but can fail completely under harsh environments. Due to the adaptation to local environments, landraces can constitute a rich source of genetic diversity. Future barley breeding might rely on such landraces in order to broaden up the genetic basis of the breeding materials and to perform better under extreme and frequently changing environments. In a large effort, we developed a purified barley landrace collection consisting of 1491 spring landraces adapted to a wide range of temperate climate conditions. In this study, we present data from 263 two-rowed landraces which were genotyped using the 9K Infinium iSelect markers. Field trials were conducted in 2013 and will be conducted in 2014. Genome-wide association studies were computed in order to detect candidate loci for yield and yield-related traits. Significant associations will be presented and discussed.

Selective study collection of *Sorghum* adaptability and quality in different regions of Kazakhstan

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Natural conditions of different regions of Kazakhstan is sharply continental, deficiency of moisture and high temperatures during the growing season requires introduction in the production not only drought-resistant, but also salt tolerant crops with high and stable yield of green mass and grain. One such valuable fodder, technical and food crop is sorghum.

Currently approved for use in the Republic of Kazakhstan grain varieties 5 and 6 varieties of sweet sorghum, most of which landraces. Selection of new drought-and salt-tolerant varieties of sorghum almost underway, which bring to the need to mobilize the global gene pool of sorghum to solve the problem of effective use of dry and saline lands in Kazakhstan.

According to the project International Center for Biosaline Agriculture (ICBA) in the conditions of the southeast, south and southwest of Kazakhstan tested genotypes of sorghum from ICRISAT, as during the spring sowing, and in summer sowing . The results of these studies for each region selected the most suitable genotypes : the southeast - ICSV 93046, ICSSH 58, ICSR 93039 and SPV 1411; southwest S35, SPV 1411, ICSV 93046, ICSR 93034 and ICSSH 58; south - ICSV 93046 and SPV 1411. Productivity of green mass during the spring sowing, depending on the varieties studied ranged 49.0-73.5 t/ha in the conditions of the southeast, 41.9-46.3 t/ha - southwest, 39.8-44.4 t/ha - southern of Kazakhstan. Productivity of green mass in summer sowing in the southeast and southwest of Kazakhstan ranged 27.67-38.77 t/ha and 27.1-38.5 t/ha, respectively. Biological grain yield grain sorghum genotypes studied to 2.8 t/ha.

Collection of sorghum protein content characterized by 11,0% to 17,2%, sugars from 17,2% to 20,8%, 7,0% of amylose to 28,7% compared to local standards Kiz 7, Kiz 94 and Zhetysu 1.

For the regions of Kazakhstan developed technology package for the cultivation and use of sorghum. Of genotypes tested by the method of individual selection and adaptive forms of selected patent pending on sugar and grain sorghum for use in the production of different regions of Kazakhstan.

Session III

Abiotic Stress Resistance

Genetic markers and landrace in spring wheat breeding for drought tolerance

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As Erythrospermum-841 remains one of the oldest cultivars (1915, landrace, Ashkhabad, researched as a spring and winter (State Tria of Variety, Moscow, 1942) with very high tolerance to drought and salinity, the application of molecular markers can be very useful in studying of its unique abiotic stress tolerance characteristics. Erythrospermum-841 had the greatest intervarietal genetic polymorphism and is probably the most genetically distant from all other Kazakh spring bread wheat cultivars studied.

Intervarietal polymorphism of the SSR markers was evaluated for wheat varieties from Kazakhstan. The cultivar Erythrospermum-841 was represented by plants having PCR products of two unique SSR markers (Ьarc0013 and barc0007), which were found only in this but not in any other cultivars studied. This is an indication of the unique origin of the variety, particularly for the chromosome 2BS.

The gene controlling the plant vernalization is represented by different dominant alleles of the spring type and one recessive allele of the winter type development. All cultivars, except for Erythrospermum-841, had one or more dominant alleles controlling the spring type of plant development. In contrast, only plants of the Erythrospermum-841 had a unique recessive allele, which can be specific to the winter type development of wheat. All studied plants of Kazakhstanskaya-19 and Erythrospermum-841 cvs had a recessive allele of vrn-Bl, typical for the winter type development of wheat. All studied plants of Erythrospermum-841 had a dominant allele of Vrn-Dl, typical to the spring-type. At the same time, all plants of the remaining varieties had recessive alleles of vrn-Dl, specific to the winter-type development of wheat.

Evaluation of wheat (*Triticum aestivum*) prebreeding germplasm for frost tolerance by a genome-wide association mapping approach

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Frost tolerance in plants is decisive to increase yield security but molecular and genetic background for this trait is still poorly understood. The aims of the present study are (1) to generate information about genetic variation for frost tolerance in a panel of 276 winter wheat accessions and (2) to find molecular markers closely linked to the trait of frost tolerance. Phenotyping of a panel of up to 360 accessions has been performed at several locations in Germany and Russia. Highly significant differences between locations but also between tested genotypes were observed. The genotyping was done employing ILLUMINA infinium iSelect 90k wheat chip. The chip carries a total of 81.587 valid and functional SNPs. After the first selection we have the possible number of 38.052 polymorphic markers for the subsequent analysis. For the population structure analysis we ran the software 'Structure' using 249 of the known markers. The Q-matrix for three groups was the best option and will be used for further analysis. We validated this result using an evolutionary tree calculated by the software 'PAUP' that also showed three clusters. The detailed analysis shows three subgroups of North American, Russian and North and Middle European samples. Further, we inferred the kinship matrix using first the subset of 249 markers and second all mapped markers in 'Tassel 2.1.' This will be used in MLM models as a random effect. First results of model comparison yield highly significant associations (LOD>3).

Mutation induction in *Sucrose synthase 1* to study cold acclimation in winter wheat

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Cold is one of the main abiotic stresses causing winterkill in winter wheat. Freezing tolerance is associated with the occurrence of a cold-hardening which is triggered by the induction of cold responsive genes after exposure of plants to low non-freezing temperature for certain periods of time. Fourteen candidate genes with known homologies were identified as being differentially expressed (presence/absence) between cold acclimated and non-acclimated crown and leaf tissues of two winter wheat lines ('5899-16' and '5450-1') using cDNA-AFLP procedure. TILLING population of the same two winter wheat lines was further developed in order to create mutant forms of the candidate genes to verify their role in freezing-tolerance formation. Firstly, we optimized the dose of a mutagen EMS to achieve substantial mutation rate while avoiding serious defects in germination and plant development. The most appropriate concentrations of EMS solution for the two winter wheat genotypes were determined and a total of 2147 M2 lines were produced. Exon 8 of the identified differentially expressed *Sucrose synthase 1* (*Ss1*) gene was chosen for mutation detection by High Resolution Melting (HRM) analysis in wheat TILLING M2 population. A total of 75.7 kb of DNA was screened resulting in an overall mutation density of one mutation per 37.8 Kb in the population. Two novel alleles of *Ss1* gene were identified, of which 1 was silent and 1 nonsense (premature stop codon) mutation. qPCR analyses were performed to estimate how these mutations affect the expression level of *Ss1* gene in crown and leaf tissue during cold acclimation. Putative knock-out mutant M631 had significantly lower relative expression of the *Ss1* gene in non-acclimated leaves as well as in crowns and leaves collected at 2, 4 and 6 weeks of cold acclimation compared with the wild type winter wheat line '5899-16' samples. Further work will estimate the effect of the mutation on the freezing tolerance in winter wheat.

Screening of rice genotypes under stress condition on the basis of SSR markers and root seedling traits

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Twenty different diverse rice germplasm lines were used to determine the relation between various traits of rice two experiments were performed under normal and water stress conditions in Randomized Complete Block Design. The diverse parents were selected based on various genotypic and phenotypic seedling traits. These traits were studied by using Correlation and Principal Component Analysis (PCA). The mean value of each genotype for the entire root shoot traits were analyzed at 1% and 5% level of significance and the highest correlation was found in root length and shoot length (r= 0.856, r= 0.896) and shoot length and root number (r=0.825, r=0.818) lowest correlation between root dry weight and shoot fresh weight (r= 0.484, r=0.591) and shoot dry weight and root fresh weight (r= 0.457, r=0.549) in stress and normal condition respectively. The principal component exhibited more than one eigen value considered to be more important due to variation. In normal condition PC1 and PC2 for root length and shoot length and in stress condition PC1 and PC2 same for root length, shoot length having more than one eigen values showed variability 76.2% and 78.5% respectively. Different SSR markers were used to study the genetic diversity and screening of the rice genotypes for making and developing of new breeding lines. The mean number of alleles per locus was 3.70; showing average number of polymorphism information content was 0.500. A total of 62.9 alleles were also identified from the microsatellite marker loci. The overall objective of the study was to screen out the diverse parents on the basis of different SSR primers and morphological seedling traits that could be used in future cross breeding program for the making of new rice varieties equally beneficial for the farmers and scientist community.

Detection of allelic diversity in genes involved in frost tolerance in bread wheat (*Triticum aestivum* L.)

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Low temperature is one of the most important limiting factors in wheat cultivation especially in regions of Eastern Europe and North America. Therefore, an association mapping approach based on candidate genes is conducted in order to identify polymorphisms involved in differences in frost tolerance which may facilitate efficient marker based breeding in the future. Frost stress affects plant growth and productivity by causing cellular damage, dehydration and reduced metabolism. For the identification of frost tolerance alleles five groups of candidate genes were analysed, i.e. (i) vernalization response genes (*Vrn*), (ii) tandem duplicated C-repeat binding factors (*CBF*), (iii) 5A cold inducible genes i.e. *Triticum aestivum* cold-regulated gene (*Tacr7*), defective embryo and meristem genes (*Dem*) and calcium-binding EF protein (*Cab*), (iv) members of the cold-inducible dehydrin (*Dhn*) gene family, and (v) photoperiod response (*Ppd*) genes. Using genomic resources available in wheat a set of 131 primer pairs was developed. It turned out that 81 amplicons corresponding to 23 candidate genes were specific and correctly localized to chromosomes (62%). 46 amplicons and five present/absent polymorphisms were analysed in a set of 24 genotypes up to now. 42 out of 46 amplicons were successfully sequenced (91.5%). In total 20 genes (87%) were sequenced in both directions, recovering the full fragment length. Sequence analysis of 20 genes revealed SNPs in 14 genes, in one gene only InDels were recorded, while in eigth genes both types of polymorphism were detected. The number of detected haplotypes per gene was varying from 2 to 5, while haplotype diversity (Hd) was between 0.2355 and 0.7312. The total number of detected polymorphisms was 129, out of which 112 were SNPs and 17 were InDels. Currently, candidate genes are sequenced in a broader set of 96 genotypes. The data of these 120 genotypes will be used for the first association analysis on phenotypic data obtained in Germany and Russia. In the end respective association genetics studies will be based on the analysis of 260 genotypes. The results of the SNP association on frost tolerance will be converted into KASP (Competitive Allele Specific PCR) or pyrosequencing markers suitable for marker assisted selection.

Usability of selection indices of durum wheat (*Triticum durum* Desf.) genotypes in Hungary

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Most climate scientists predict drier, more variable weather due to climate change, and there is also increased attention on global food security (fuelled by the estimates of 9 billion people by 2050), therefore crop plants resistance against drought is getting more important in the breeding programs. The main objective of this work was to evaluate 19 durum wheat genotypes based on grain yield (GY) and drought tolerance indices under rainfed and irrigated condition and to validate the applicability of indices in prediction of yield in Hungary, during the 2011-2012-2013 cropping season. Unbalanced incomplete alpha lattice block design was used in three replications for rainfed experiment and two for irrigated. Based on GY drought tolerance indices i.e., stress intensity (SI), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI), tolerance (TOL), yield stability index (YSI) were calculated. Statistical analysis of the data was performed using GENSTAT 16 software. The results of analysis of variance for GY and yield components indicated that genotypic differences were highly significant ($P<0.01$). Based on each agronomic trait the response of genotypes at each condition and at each year differed. The correlation of yields between the rainfed and irrigated conditions was insignificant, consequently high potential grain yield under optimal conditions does not necessarily result in desired yield under stress conditions. This result suggests that selection based on low TOL does not result in high level of adaptability to drought. MP, GMP and STI had a positive correlation with GY under both conditions, the STI value of the genotypes varied over a narrow range within year. YSI had a significant positive correlation with GY under rainfed condition, however the YSI value depend on the stress severity. The SSI correlation with GY shows that selection for high SSI will result low yielding genotypes in irrigated condition. The outcome of this study indicates that use the STI index could be one more additional parameter in the breeding program to select high yielding genotypes under different conditions, but breeders should take into account the severity of the stress in the target environment.

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Influence of seasonal drought on yield in spring barley

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Drought is one of the major constraints for agricultural production via plant performance. Hence, it is essential to study the effects of drought on growth and development of crop plants. Response of plants to drought is complex and depends on timing, duration and intensity. Hence, the combination of high-throughput phenotyping (HTP) with non-invasive means and controlled watering would help in better understanding this complex trait. Yield reduction can be caused by early drought events via reduced number of tillers and a smaller photosynthetic active leaf area or by later drought events via decreased seed set or reduced grain filling. Our previous study focused on early seasonal drought impact on 100 two-rowed spring barley accessions for vegetative biomass development screened on an automated HTP imaging-based system. These 100 two-rowed spring barley accessions are part of Genobar collection investigated in the past for various traits (Pasam et al. 2012). In a validation experiment lasting until maturity we have now screened a subset of 26 contrasting lines (improved cultivars and landraces from 16 different countries) under two treatments (well-watered and vegetative drought stress) with 10 plants per accession to investigate if vegetative drought tolerance is connected with a higher yield. The plants were subjected daily for automated watering [90% field capacity (FC)] and imaging in the visible light spectrum. Drought was imposed by withholding watering for 18 days starting from 27 days after sowing (DAS). Plants were then re-watered at 45 DAS until maturity. Imaging data were used to extract 'digital biomass' – a pixel volume showing a high correlation to plant shoot fresh weight, for all the individual plants (IAP, Klukas et al. 2012). Plants were imaged until DAS 70 and then transferred to a greenhouse for the ripening process. This experiment allowed us to investigate the influence of vegetative biomass on several yield-related traits and the effect of drought stress in vegetative phase on shoot biomass, grain yield or harvest index – which will be presented.

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Comparison of *Triticale* and bread wheat cultivars under drought stress after anthesis

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Post-anthesis drought stress, often associated with heat stress, is a common problem in most cereal growing regions of the Mediterranean Basin. Ten triticale (X *Triticosecale Wittmack*) and bread wheat (*Triticum aestivum* L.) cultivars developed in the south-east Europe were tested under drought during grain filling to evaluate triticale as an alternative to bread wheat in such growing environments. Field experiments were carried during three consecutive growing seasons with plants defoliated 10 days after anthesis and grown with intact control plants under well-watered conditions. The cultivars were scored for 26 agronomic traits. Observed traits suggested that the higher yields under terminal drought in triticale include greater chlorophyll content in the flag leaf at anthesis, thousand grain weight,biomass per plant, higher grain filling rate and early vigorous growth. In wheat, yield benefit can be achieved from increasing biomass per plant and production per spike, wider flag leaves and greater early vigour. Almost all productive traits of triticale, except productive tillering and kernels per unit area, had less reduction due to drought stress than that of wheat including thousand grain weight (18.4 vs 23.8%), hectoliter weight (3.1 vs 3.8%), biomass per plant (24.5 vs 28.1%), harvest index (1.8 vs 3.9%), production per spike (17.4 vs 21.2%) and grain filling rate (18.7 vs 26.7%). Stress indices based on grain yield showed the better respones of triticale cultivars to post-anthesis drought conditions.As triticale often out-yields wheat also in favourable conditions, it seems to be an interesting alternative to bread wheat in variable conditions after anthesis.

RNAseq analysis of differentially expressed genes between two barley cultivars with contrasting yield under drought

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The traditional cultivars and landraces of the Spanish Barley Core Collection [1] harbour genetic variability of potential interest for breeding elite varieties adapted to Mediterranean environments. Here we compare *in vivo* gene expression profiles of two contrasting cultivars when grown under drought-stress: SBCC073, a local landrace with high yield under drought conditions, and elite cultivar Scarlett, an European variety that performs poorly with low water supply. Plants of both varieties were cultivated under different watering treatments and RNA extracted from several tissues for subsequent library preparation and high throughput sequencing of biological replicates with Illumina HiSeq 2000 instruments. A total of 1.2 billion 2x100 reads were obtained, which were subject to removal of adapters and low-quality sequences. As the only available references are the genomic sequences of European and American cultivars Morex, Barke and Bowman, reference transcriptomes of SBCC073 and Scarlett were also sequenced and assembled using Trinity [3]. After removing chimeras and other unlikely constructs, nearly 298941 transcripts were validated by stringent alignments (coverage ≥95% and identity ≥80%) to barley and other Triticeae sequences. Gene and isoform quantification was performed with RSEM [4] and differential expression analyses with edgeR [5]. We compared expression profiles between watering treatments for each assayed tissue. Differentially expressed genes and isoforms of each comparison were mapped to high and low confidence barley genes and used in posterior GO enrichment analysis (custom scripts). Preliminary results suggest a higher expression of proteins related to stress in SBCC073. We also identify the protein kinases and transcription factors present in each set of differentially expressed genes and isoforms using software iTAK [6]. This study reveals molecular details of the adaptation of Mediterranean barley cultivars to drought.

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Effects of ambient temperature on the plant developmental patterns of wheat (*Triticum aestivum* L.)

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Ambient temperature plays important role in determining the plant developmental patterns in cereals, when they are under inductive conditions. The genetic components of temperature sensing, however have not been dissected yet. In order to evaluate the natural variation in the ambient temperature reactions in wheat, 21 wheat cultivars of various geographic origins were included to a controlled environmental experiment in which the effects of three temperature regimes – 11°C, 18°C and 25°C – applied under long photoperiod and after vernalization were evaluated via measuring the phenological properties of the wheat genotypes in details. In this temperature range, the average thermal time required for a given developmental phase increased parallel to the higher temperature. This was accompanied with an increase in the final leaf number and with a decrease in final plant height and tillering. There were significant variations between the temperature responses of the wheat genotypes. Based on the ranks of heading dates under the three temperature regimes, 38% of the cultivars were relatively insensitive to the ambient temperature, while the remaining genotypes represented various combinations of temperature sensitivity.

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Breeding for novel traits for adaptive tolerance to hostile soils

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Abiotic stresses such as low water availability and high salinity are the major causes of cereal crop yield loss and significantly impact on sustainability. Over 800 million hectares of land throughout the world are salt affected, either by salinity (397 mha) or the associated condition of sodicity (434 mha). Wheat is an important cereal crop and being a staple food grown in hostile environments like soil salinity/alkalinity and drought conditions. However, wheat classified as glycophytes (salt sensitive), yet it is more salt tolerant than other cereal crops such as rice and so are good models for studying salt tolerance in cereals. There are five possible ways, which can be used to develop salt tolerant genotypes: 1) develop halophytes as alternative crop; 2) use interspecific hybridization to introgress tolerance into available cultivars; 3) exploit the variation already present in existing germplasm; 4) generate variation within existing genotypes by using recurrent selection, mutagenesis or tissue culture and 5) breed for yield rather than tolerance. In order to identify genotypes tolerant to harsh soil conditions, thirty-six genotypes were evaluated at hot spots (Hisar, Faizabad, Coochebehar and Degraj) under natural conditions where pH ranged from 7 – 9.5 and normal soils at Karnal. The initial data recorded so far revealed that the harsh conditions affected the performance of genotypes. Germination at hot spots was reduced in all the genotypes by 10 – 50%. Average germination under normal soils was 77% and at Hisar was 51%. The coleoptile length ranged between 3.1 to 3.9 cm under normal soils and 4.4 to 10.1 cm under harsh soils. However, a few genotypes (CBW 38, DBW 51, K 0307, KRL 3-4 and NW 4092) had significantly longer coleoptiles. Plant height measured at 45DAS was reduced by 10 – 50% under harsh soils in most of the genotypes except DBW 17, KRL 210, MACS 6222, RAJ 4229 and WH 1105. The data to be recorded on yield related traits will reveal whether the gains in initial growth in some genotypes is translated into grain yield/ or not.

Identification of QTLs for drought stress-related physiological traits by genome wide association studies in barley

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Drought stress during spring and summer can result in severe yield losses in barley. Climate models predict an increase in drought periods in Germany. Therefore, tolerance to drought stress will be an important goal in barley breeding in the future. As drought stress tolerance is difficult to select in the field, genomic selection may be an option. As a prerequisite to include this trait in the genomic selection procedure, a reliable high-throughput screening system for drought tolerance has to be developed to phenotype the calibration set.

To achieve this, in a first step 63 six-rowed winter barley cultivars, which have been analysed in association genetics studies, already, were grown under controlled conditions in a growth chamber up to the four leaf stage. Stress was applied by wilting single leaves or leaf disks or by putting leaf disks on a PEG medium for 48h. On wilting leaves the relative water content as well as the chlorophyll fluorescence was estimated. On leaf disks the osmotic adjustment, the content of soluble sugars and free proline as well as the cell membrane stability were analysed.

For all six physiological parameters significant differences between the stressed and non-stressed plants were detected indicating a successful stress application. The results also revealed significant differences between these genotypes after stress application which is a prerequisite to score these traits as indicators for drought tolerance.Based on correlations to two-year yield data obtained on these genotypes in rain-out shelter trials for drought tolerance and pot experiments for early leaf senescence, the three traits relative water content, chlorophyll fluorescence, and osmotic adjustment were selected for the analyses of 780 six-rowed winter barley breeding lines and varieties (calibration set).

The phenotypic data of this calibration set obtained from those analyses, and the genotypic data generated by the Illumina 9k iSELECT chip were used for genome wide association studies. Genomic regions significantly involved in chlorophyll fluorescence under early drought stress conditions were predominantly identified on barley chromosome 2H, whereas genomic regions involved in relative water content and osmotic adjustment were identified on chromosomes 3H, 5H, 6H and 7H.

Quantitative trait loci analysis of barley RIL lines derived from hybrids between European and Syrian cultivars differentiated in tolerance to water deficiency

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POLAPGEN-BD (www.polapgen.pl) project is the largest coordinated plant genomics project in Poland. It concerns drought resistance in cereals, investigated in spring barley as a model plant. The project will provide knowledge on the variation of morphological, anatomical, physical and physiological features of spring barley grown in partially controlled conditions and subjected to drought stress, in conjunction with the response at molecular level (studied by several research partners). Materials for the studies cover – among others – a population of 100 recombinant inbred lines derived by single seed descent technique from the cross between Lubuski (European) and Cam/B1/CI08887/CI05761 (Syrian – adopted to water shortage) cultivars. They were examined in the field experiment carried out in the complete randomized design with three replications and also phenotyped in the greenhouse under optimal and drought conditions. Drought stress was applied at the three leaves stage (phase 13 in the BBCH scale) during 10 days and flag leave stage (phase 37 in the BBCH scale) during 14 days. Soil moisture was kept at 2.2 and 3.2 pF in optimal and drought conditions, respectively. Yield-forming traits were observed in lines and their parental genotypes. Phenotyping was related to 25 characteristics associated with the spike morphology, plant architecture, grain and straw yield, as well as dates of achieving selected developmental stages of barley. It was found that water shortage affected most of the analysed traits. The population was also genotyped with microsatellite (SSR) and single nucleotide polymorphism (SNP) markers. On this basis a genetic map was constructed and QTLs determining the yield potential were identified. A high-density linkage map allowed finding genetic markers related to observed drought effects.

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Participatory plant breeding for food security and adaptation to climate change: The case of barley

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Strengthening food security of rural populations, reducing barley vulnerability to pathogens and abiotic stresses and prepare the farmers to meet the new challenges linked to climate change is one of the major goals in the research system in Ethiopia. Increasing seed production of barley with high resilience and good productivity from indigenous and traditional varieties, through participatory and evolutionary selection and introduction of agricultural practices is the major focus in barley improvement program to enhance the interactions between genetic improvement and agronomy. To this end, a participatory plant breeding (PPB) was conducted at six locations in Ethiopia (4 at Holetta and one each at Bekoji and Sirinka), The PPB at Holetta targeted a farmer's research group with 180 members, mainly composed of women farmers. Barley varieties composed of landraces and breeding lines and elite varieties were evaluated in selected farmers' fields following three rotation systems, namely barley after potato; barley after tef and barley after fallow. The barley varieties were evaluated by a group of farmers at the different crop growth stages and after post-harvest on the grain physical characteristics from their own perspectives. It was revealed that in some cases there is a remarkable coincidence between grain yield and farmers' preferences. Of particular interest varieties like HB 1307 that is found among the top 10 in all six locations, and EH 1493/F6.32H.3 resulted in the top 10 in all locations except Holetta.. The various discussions made in the process of participatory variety evaluation in the PPB program confirmed that farmers had already good knowledge and understanding on climate changes associated to their farming systems. Based on these results, and to meet the expectations raised during the participatory evaluation of the project, a preliminary list of varieties were identified for further evaluation by making available to farmers the seed of preferred varieties. The varieties chosen are those that combine high grain yield and a high farmers' score with good adaptation.

The wheat height reducing genes affect plant responses to drought

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Soil water deficit is currently a leading environmental challenge for the developing plant. The adverse effects of drought on plant growth and yield are mostly associated with the accompanying osmotic and oxidative stress, photosynthesis limitations and changes in leaf anatomy. Plant growth and physiological reactions to stress depend on phytohormonal balance and signal transduction. The wheat gibberellin-insensitive *Rht* (reduced height) genes encode defective DELLA proteins with aberrant gibberellin responses and thus inhibit growth. The present study proposes an additional effect of the della *Rht* genes on stress tolerance in wheat. Seedlings of *Rht-B1b* (semi-dwarf) and *Rht-B1c* (severe dwarf) near-isogenic lines in background of the bread wheat cultivar April Bearded were subjected to soil drought and their physiological responses were compared to the reactions of the tall control, *Rht-B1a*. The stress was imposed by six-day-long water deprivation corresponding to 35 % of soil full moisture capacity compared to 60 % in optimally watered controls. Lines *Rht-B1c* followed by the *Rht-B1b* were more tolerant than the tall counterpart as evidenced by less damaged membranes, higher accumulation of compatible solutes, enhanced antioxidant defence, less disturbed photosynthetic functions, and less affected leaf anatomy. These results suggest a pleiotropic effect of the *Rht-B1* gene associated with modified gene expression under drought. The stress induced accumulation of defective gene products in plants with alleles of different size reductions possibly elicits divergent physiological responses and could enable better adaptation to the environmental changes.

Impact of arbuscular mycorrhizal fungi on drought stress tolerance of wheat

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Wheat (*Triticum aestivum*) is one of the most important crops worldwide, used mainly for human nutrition as well as for feeding animals. In future, plant breeders increasingly will face new challenges, because of changes in growing conditions. The expected increase of drought periods, in particular early summer drought, will result in remarkable yield and quality losses. Until now, the most promising approach to reduce the negative impact of drought stress is the identification of stress tolerant wheat genotypes. A new approach to increase drought stress tolerance of wheat is the identification of genotypes, which are able to generate symbiosis with mycorrhizal fungi. It is known that mycorrhiza symbioses are beneficial to many plant species under drought stress conditions by increasing water and nutrient uptake leading to an increased yield. Therefore, the objective of the project was the identification of genotypic differences in mycorrhization and of wheat genotypes which benefit significantly from mycorrhizal colonization under stress conditions.

To achieve this, a set of 103 wheat genotypes was investigated under drought stress conditions in pot experiments in order to detect genetic differences in drought stress tolerance of these genotypes, their ability to form a symbiosis as well as to get information on the impact of the symbiosis on yield and yield parameters.

Genotypes were grown in a pot trial under glass house conditions in a drought stressed and a control variant with and without mycorrhization in three replications and yield and yield components were assessed.

Root colonization by mycorrhizal fungi *Glomus intraradices, Glomus etunicatum* and *Glomus claroideum* was analyzed by PCR analysis and an ink vinegar stain of root segments. The majority of wheat genotypes was colonized by one or more mycorrhizal species of which *G. intraradices* was predominant. Differences in mycorrhization between genotypes under drought stress conditions could be detected by light microscopical techniques. Furthermore, significant differences of yield were found between the mycorrhizal and non mycorrhizal treatment under drought stress conditions. In parallel, genotyping using the 90k iSelect chip was conducted to identify QTLs involved in mycorrhization and drought stress tolerance via genome wide association genetics studies (GWAS).

Impact of suboptimal doses of nitrogen on wheat growth at early developmental stage

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Nitrogen (N) is a significant factor limiting plant growth, and plants frequently encounter with different nutrition conditions in their natural habitats. Deficiency as well as excess of N in the environment seriously interfere to the natural metabolic balance of plants and have many negative effects on the physiology and growth of plants. In the present work is focused on investigating the effects of (sub)optimal concentrations of nitrogen (0-35 mM) on wheat plants (*Triticum aestivum* L. cv. Genoveva) grown hydroponically. Data show that N availability correlates well with biomass growth and content of photosynthetic pigments. Further, in roots it affects membrane lipid peroxidation level, while in shoots altered levels of free proline were detected indicating to different defense strategy in the two organs under different N conditions. Reactive oxygen species are also generated under extreme N conditions. The obtained data could be applicable for optimization of fertilization strategy for sustainable agriculture.

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Exploring genetic response to nitrogen supply in wheat

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Nitrogen (N) is a major nutrient and is needed to attain high grain yield in low-yielding environments. Improving varieties with high nitrogen use efficiency (NUE) is of interest to plant breeders in modern agricultural systems. In Mediterranean climates with low rainfall and high temperature during grain filling, developing cultivars capable of using N efficiently would be economically beneficial. This project aims to detect the genetic loci underlying nitrogen response under different nitrogen fertilizer regimes in a bread wheat population derived from a cross between two local Australian cultivars (RAC875 × Kukri). In total, four NUE field trials in South Australia in 2011 and 2012 at two sites, have been carried out. The estimated genetic efficiency and responsiveness effects were calculated using BLUPs for the 180 double haploid lines under three nitrogen treatments at all sites. There was genotype by environment by treatment interaction across the sites and also good transgressive segregation for yield under different nitrogen supply in the population. We detected some significant QTLs associated with the efficiency at different levels of nitrogen application across the various environments and years. It is also possible to select favourable lines showing positive N response based on the rankings of their BLUPs within a trial. Dissecting the complexity of the N effect on yield through QTL analysis comprises the first stage in cloning genes underlying the loci affecting NUE in wheat.

Sorghum genotypes differ in their response to free air carbon dioxide enrichment under drought

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Maize has a high biomass production and therefore is used as an energy crop for the production of methane as renewable energy source. Given the increase in temperature and the decrease in summer precipitation as predicted by the IPCC, sorghum could be an alternative energy crop in the future due its better drought tolerance. Therefore we analysed the growth response of maize and different Sorghum-genotypes under present and future climatic conditions, i.e. under increased atmospheric CO_2 concentration and severe summer drought. Two CO_2 (ambient and approx. +210 ppm above ambient) and water treatments (WET and DRY) were provided by the operation of FACE (free air CO_2 enrichment) systems and rain shelters, respectively.

In the first year, the late start of the field experiment and the rainy summer made the drought treatment difficult and plant growth was almost similar among the two watering regimes. However, in the second year plants grown under rain shelter received only half the amount of water of the WET treatment and growth was significantly affected by water supply. Maize had a higher biomass production than sorghum under both watering regimes. CO_2 enrichment increased growth of all C_4 plants under DRY conditions, but not under WET conditions. The sorghum-genotypes differed in above ground biomass production and there was also a significant interaction between genotype and water supply. The mitigation of drought effects on plant growth by CO_2 enrichment differed between all plants studied.

Phylogenetic analysis of SOS (*Salt Overly Sensitive*) genes in different species of *Triticum* and *Aegilops* genera

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Salinity stress is one of main factors affecting cereal yield around the globe. Salt tolerance is an agronomical important trait to analyze and transfer in commercial cultivars to improve them. Enhanced salt tolerance can be achieved by overexpression several genes that regulate ion homeostasis, such as SOS (*Salt Overly Sensitive*) genes which represent key components in salt tolerance. Ion homeostasis is mediated mainly by the SOS signal pathway, which consists of three main components. SOS1 encodes a plasma-membrane Na+/H+ anti-porter that plays a critical role in sodium extrusion and in controlling long distance Na+ transport from the root to shoot, SOS2 encodes a Ser/Thr protein kinase while SOS3 encodes an EF-hand Ca^{2+}-binding protein that functions as a calcium sensor for salt tolerance. Salt stress elicits a transient increase of Ca^{2+} that is sensed by SOS3. In present work, primers for these genes have been designed and used to identify their presence in different *Triticum* and *Aegilops* species characterized by different genomes. For each gene, more primers have been designed to amplify all the conserved regions of the gene; the sequences obtained have been multi-aligned and analyzed by means of statistic softwares in order to obtain the genetic distances among the different species and identify mutations and INDEL. The species analyzed comprised *Triticum turgidum* (tetraploid, genome AABB) subsp. *durum*, *carthlicum*, *polonicum* and *turanicum*, the three cultivated hulled wheats species, *T. monococcum* L. (diploid, genome A), *T. dicoccum* (tetraploid, genome AB) and *T. spelta* (hexaploid, genome ABD), and their wild putative progenitors such as *Aegilops speltoides* (diploid, genome B), *Ae. tauschii* (diploid, genome D), *Ae. sharonensis* (diploid, genome S) and *T. urartu* (diploid, genome A). The SNPs and INDEL mutations present in SOS genes have been analyzed, the results show that these genes are conserved within the same genus, flatly differing between *Triticum* and *Aegilops* species. The differences found are in accord with botanical classification of these species. Considering the *Triticum* genus, the sequences result highly conserved, clearly grouping in the *turgidum* sub-species (*durum*, *carthlicum*, *polonicum* and *turanicum*).

‘Stabilstroh’: A new hope for lodging resistance

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Lodging, the state of permanent displacement of the tillers from their upright position, causes decrease in grain quality and furthermore increases the costs of harvesting. Therefore lodging resistance is one of the main breeding objectives in crop production, especially in rye (*Secale cereale* L.), where yield losses due to lodging can be as high as 75%. ‘Stabilstroh’, a recently identified genotype of rye, not only has the best lodging resistance, but simultaneously it is characterized by the longest tillers among the German cultivars of rye hybrids.

To identify genetic traits responsible for lodging resistance histological and ultrastructural studies were performed on the basal internodes of segregating F2 population (‘304/1’) and its parental lines: ‘ms135’ („Stabilstroh”) and ‘R1124’ (wild type). Analyses of tissue distribution, cell size, and cell wall thickness using Light Microscopy, Scanning Electron Microscopy, and Transmission Electron Microscopy revealed that the ‘Stabilstroh’ genotype has a significantly higher ratio of the thickness of sclerenchyma to parenchyma tissues, while both: sclerenchyma cell walls and inner periclinal cell walls of the epidermis, were significantly thickened in comparison to the wild type. Furthermore a standard phloroglucinol staining for lignin confirmed its higher content in ‘Stabilstroh’ tillers. Invaginations of the stem, an important factor enhancing mechanical stability, were also much more abundant and pronounced in the genotype ‘Stabilstroh’. It is expected that all these traits are significantly improving lodging resistance and biomass production in rye.

Currently the F_2 population and its parental lines are being genotyped using microsatellite (SSR) markers. The quest for QTLs (Quantitative Trait Loci) for improved lodging resistance will be based on the inheritance of traits affecting mechanical stability of tillers, as: stalk invaginations, thickness of cell walls, lignin content, and sclerenchyma/parenchyma ratio.

Engineering phenology genes in wheat for higher yields in water-limited environments

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Wheat (*Triticum aestivum* L.) is the most widely grown grain crop in Australia and throughout the World, and yield is often limited by environmental stresses such as drought. Australia's wheat crop is grown mainly under rain-fed conditions, and yield losses vary with the timing and intensity of drought. Phenology genes play an important role in adaptation to a particular environment by modifying duration of crop developmental phases, and hence breeders have the opportunity to optimize developmental phases for differing environments by manipulating the allelic composition of the phenology genes. Vernalization (*Vrn*), photoperiod (*Ppd*) and autonomous earliness per se (*Eps*) genes are the components of wheat phenology which interact in the flowering pathway to determine the development and time of maturity of wheat.

The aim of this study is to determine the most appropriate allelic combinations of *Vrn1* and *Ppd* genes that may be appropriate for various growing regions in water limited environments. An experiment was conducted both in drought and control condition with a double haploid (DH) population segregating for all three Vrn1 loci. Variation in their allelic combination had a significant effect in flowering and maturity, where we observed 40 and 20 days variation in heading and physiological maturity, respectively. Heading date was positively correlated with both the spike length and the seed number per spike in control treatment but negatively correlated with the seed number in drought condition, which might be due to the seed abortion or less spikelets development. The number of dominant alleles (of three *Vrn* genes) present in a given DH line was positively correlated with the early flowering and maturity, but the seed weight was reduced, possibly due to a reduction in the duration of grain filling. Early flowering could be utilized to optimise yield in the environments where terminal drought occurs. The presentation will also include the implications for various cropping systems.

Estimation of quantitative genetic parameters for pre-harvest sprouting resistance in wheat under laboratory and field conditions

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Pre-harvest sprouting in wheat can cause a reduction in yield, test weight and grain quality creating large financial losses to producers. Tolerance to pre-harvest sprouting is therefore a desirable trait of a cultivar in conditions of rainy weather before harvest. The objective of the present study was to estimate variance components and heritability of resistance to pre-harvest sprouting in wheat under field and laboratory conditions. Field trials including 25 winter wheat varieties were conducted during three successive growing seasons in total of eight environments (location-year combinations) following a randomized block design in two replications. Pre-harvest sprouting resistance of the studied varieties was estimated by measuring the level of dormancy in laboratory germination tests with threshed grains, whereas field sprouting damage was estimated by the Hagberg falling number (HFN) test as well as by the percentage of sprouted grains in the field (field sprouting). All tests were conducted on samples taken at harvest ripeness (Time 1) and 14 days after harvest ripeness (Time 2). The environmental component of variation accounted for 1 to11 % of the total variance for laboratory germination tests. For HFN test it accounted for 28% and 70% for Time 1 and Time 2, respectively, and for field sprouting it accounted for 52% of the total variance. The proportion of the genetic component of variance in the total variance ranged from 65% to 83% for germination tests and from 15% to 40% for HFN test, whereas it accounted for only 7% of the total variance for field sprouting. The magnitude of genotype x environment (G x E) variance was similar for germination tests and the HFN test (10 -16% of the total variance) whereas G x E variance for field sprouting accounted for much higher amount to the total variance (35%). Heritability estimates on an entry mean basis varied from 0.96 to 0.98 for germination tests, from 0.90 to 0.93 for HFN tests and was only 0.61 for percent of sprouted grains in the field. Phenotypic correlations among tests varied across environments.

Exploiting phenotypic variability in thermal tolerant x susceptible RIL population

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Wheat, a climate sensitive crop, is grown on 28 -29 million hectare and about 13.5 mha of area faces thermal stress. It is predicted that increase of 1^0C in temperature will result in 4-5 million tonnes of loss in wheat production. The heat stress resulting from delay in sowing by one month can lead to about 20-30% loss in grain yield. Under late sown conditions, the crop gets exposed to temperatures above 35°C at the time of grain development. Tolerance to heat stress is complex phenomenon and controlled by multiple genes imparting a number of physiological and biochemical changes and no single trait explains the mechanism of heat tolerance. Thus, the use of correlation and co-segregation analysis, and molecular marker techniques in genetic stocks with different degrees of heat tolerance are promising approaches to dissect the genetic basis of thermo-tolerance and for this purpose recombinant inbred line population is generally used. Although both phenotypic and genotypic data is obtained for the purpose, rarely phenotypic data is used to elucidate the better performing lines, which are transgressive segregants. One hundred and seventy-two RILs derived from RAJ 3765 (heat tolerant) and P 11632 (heat susceptible) were planted at Directorate of Wheat Research, Karnal in three consecutive seasons under two planting conditions, normal and late sown representing control and heat stressed environments. To determine the impact of heat on RIL lines, HSI was estimated. The objective was to assess these lines for heat tolerance and to identify superior lines. Considerable variation was observed for grain yield (GY), thousand grain weight (TGW), grain fill duration (GFD), chlorophyll content index and chlorophyll fluorescence. The lines were grouped into four categories based on the HSI, the number of heat tolerant lines varied from 18 to 42% in the population. When compared with grain yield under heat stress, lines 145 and 161 were numerically superior to both parents line no. 145 was also best for HSI of grain yield. The results demonstrated that it is possible to obtain lines that perform better for yield and yield related traits in heat stressed environments.

Abscisic acid flux alterations result in differential ABA signalling responses and impact assimilation efficiency in barley under terminal drought stress

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The plant hormone abscisic acid (ABA) is a central player in plants response to drought stress. How variable levels of ABA under short-term versus long-term drought stress impact assimilation and growth in crops is unclear. We addressed this through comparative analysis, using two elite breeding lines of barley that show senescence or stay-green phenotype under terminal drought stress and by making use of transgenic barley lines that express *AtNCED* (*Arabidopis thaliana* 9-cis-epoxycarotenoid dioxygenase) coding sequence or an RNAi sequence of *ABA8'-OH* (ABA 8'-hydroxylase) under the control of a drought-inducible barley promoter. The high levels of ABA and its catabolites in the senescing breeding line under long-term stress were unfavourable for assimilate productivity whereas these levels were not perturbed in the stay-green type that performed better. In transgenic barley, drought-inducible *AtNCED* expression afforded temporal control in ABA levels such that the ABA levels rose sooner than in wild-type (WT) plants but also declined, unlike as in WT, to near-basal levels upon prolonged stress treatment due to down-regulation of endogenous *HvNCED* genes. The repression of ABA catabolism by down-regulation of *ABA8'-OH* led to lower ABA flux during the entire period of stress. These transgenic plants performed better than the WT under stress by maintaining a favourable instantaneous water use efficiency and better assimilation. Gene expression analysis, protein structural modelling, and protein-protein interaction analyses of the members of the *PYR/PYL/RCAR*, *PP2C* and *SnRK2* family identified specific members that could potentially impact ABA metabolism and stress adaptation in barley.

Combined effect of the drought and the atmospheric CO_2 concentration on the water use properties of winter wheat

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In Central Europe drought is the most important limiting factor for autumn-sown cereals. Due to the decline in groundwater, it is vital to make efficient use of groundwater and to promote less water-demanding forms of crop production. Water use efficiency can only be increased if cultivars with satisfactory water management traits are grown, so that they can exploit water reserves of the soil even if drought occurs during the vegetation. To this end, water consumption (WU) and water use efficiency (WUE) of winter wheat genotypes were investigated in a greenhouse experiment. Plants were grown either with optimum water supply or with simulated drought in one of two phenophases, shooting and heading. Measurements were made on yield parameters, phenological traits and water use parameters of plants. This experiment was carried out in greenhouse chambers using the same climatic conditions but atmospheric CO_2 concentration was regulated. 400 ppm concentration was used as control and elevated levels were 700 and 1000 ppm, respectively. Water use efficiency was calculated by dividing the grain yield by the water used during the vegetation. Significant differences were determined investigating the influence of water shortage on the water use efficiency of plants during the vegetation but also meaningful alterations were found among the drought and CO_2 sensitivity of varieties examined. Elevated CO_2 concentration improved the water use efficiency but there was found some differences among the genotypes that could be followed not only by the control but also by the drought treated plants. Simulated drought stress by the shooting decreased the WUE compared to the well watered plants but there were meaningful differences among the varieties at the different CO_2 levels. The enriched CO_2 resulted in a significant increase of WUE under stress condition simulated by the heading compared to the plants grown under atmospheric CO_2 level.

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Responses of different winter barley genotypes to water shortage under elevated CO_2

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As the result of global climate change with rapidly increasing atmospheric CO_2 concentration and changes in rainfall patterns, the future production of crops is faced with the challenge of extreme variations in soil water conditions accompanied by a much higher supply of CO_2 as a basic substrate of photosynthesis. Due to its antitranspirational effect elevated CO_2 was repeatedly shown to partly mitigate yield losses in cereals including barley caused by drought stress. However, no information about the genotypic variability of this mitigating effect in winter barley is available yet.

In order to achieve the aim of sustainable agriculture and of a minimization of yield fluctuations over years by breeding climate-smart crops, it is important to determine how effectively different genotypes are able to use the limited available water resources under future CO_2 conditions.

A selection of winter barley genotypes including four two-rowed and six six-rowed genotypes which showed different growth responses to elevated CO_2 in previous experiments were investigated for their responses to water shortage under different CO_2 concentrations in a greenhouse.

The experiment was conducted in a 2x2 factorial design and plants were grown either with optimum water supply or simulated drought during the heading at either current (~400ppm CO_2) or elevated CO_2 concentrations (~700ppm CO_2) with otherwise identical climatic conditions.

After 42 days of vernalisation, five seedlings were planted in pots containing 10,000 cm^3 of a 3:1:1 (v/v) mixture of soil, sand and humus. For plants given optimum water supplies the soil water content was maintained at 60% of the soil water-holding capacity, equivalent to volumetric water content (v/v) of 20–25%. Water deficit was simulated by water withholding for 7–10 days. Water consumption was measured by weighing the pots and replacing the water on a weight basis.

Phenological and yield parameters (tiller and ear number, plant height, aboveground biomass, grain yield, TKW; HI) were evaluated and water use efficiency (WUE; kg m^{-3}) was calculated by dividing the grain yield (kg) by the total water use during the vegetation period (WU; m^3).

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Session IV

Biotic Stress Resistance

Multispectral camera and imaging for assessment of resistance to *Fusarium graminearum*

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The multispectral imaging analysis has been developed by GEVES to assess the phenotypic resistance to Fusarium (*F. graminearum* and *F. culmorum*) on wheat, by comparison with visual disease assessment (VDA) and qPCR analysis performed by Arvalis. The aim was to find a new method of phenotyping to replace VDA currently used for the resistance assessment in the frame of VCUS studies (Value for cultivation and Use and Sustainability) for the registration in the French Catalogue. In 2013, GEVES has developed an algorithm to quantify the percentage of Fusarium damaged kernels (FDK) measured by Videometer, which records a multispectral image from successive illumination of 20 different high resolution bands, thus increasing greatly the accuracy of the colors and introducing information from the ultraviolet to near-infrared bands. This method has the advantage to be non destructive and faster unlike qPCR analyses. The first trials were inoculated by *Fusarium graminearum* and *culmorum* on CTPS wheat controls and identification of pathogens was verified by microbiological analyses.

A very strong correlation was observed between VDA (percentage of infected spikelets) at 360°C after the flowering date and the percentage of FDK at harvest maturity, measured by Videometer, with R^2=0.95, in a study carried out on 125 ears of five controls, with an analysis per individual spike. A high correlation was also founded, with the analysis of 25 spikes in bulk of these five varieties (giving about 1000 kernels/cultivar), with R^2=0.84, as well as using an higher speed system to carry the seeds with a conveyor belt.

In the case of a contamination only by *F. graminearum and culmorum,* high correlations were obtained between qPCR and Videometer with R^2=0.82, and between qPCR and visual ratings with R^2=0.91.

These analyzes will be pursued in 2014 by GEVES for the confirmation of the classification of varietal resistance to *Fusarium graminearum* on a higher number of wheat varieties, and also on the other species of cereals. A new study should be launched to develop another algorithm able to distinguish on seeds *Microdochium* spp from *Fusarium graminearum* on wheat.

Molecular identification of slow rusting leaf rust resistance genes in the Romanian wheat germplasm

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Leaf rust caused by *Puccinia triticina* reduces the wheat yield and grains quality worldwide. In the fight of wheat against *Puccinia triticina* and the fight of breeders for obtaining resistant cultivars, host resistance genes play a major role. Therefore, the identification of resistance genes in modern wheat cultivars and breeding lines, and then selection of the best resistance genes combination(s) are the first steps for a successful breeding. Markers assisted selection (MAS) allows a significant improvement of breeders' efforts. At present, among the known genes with slow rusting effect the most common are the genes: *Lr34, Lr46, Lr67* and *Lr68*.

The aim of this study was to evaluate the presence of designated slow rusting resistance genes to leaf rust in Romanian wheat germplasm, using molecular markers. An overall screening of 190 winter bread wheat cultivars and lines with functional marker, *cssfr5*, has showed presence of *Lr34* haplotype resistance in proportion of 59% (homozygous genotypes 49% and heterozygous genotypes 10%). We have observed that the percentage of *Lr34* gene is very low (10%) in genotypes obtained before 1989 and very high at genotypes obtained after 1990 (90%) Separately, the presence of other leaf rust resistance genes like *Lr68*, recently described with slow rusting effect has been found in some genotypes using STS-csGS and CAPS-cs7BNLLRR markers.

Further investigations are focused on identification of the same slow rusting genes in the new breeding material and on search of other similar genes.

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Marker assisted backcrossing for *Fusarium* head blight resistance and high grain protein content in winter wheat

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Marker Assisted Backcrossing (MAB) may be an efficient strategy for plant breeders to incorporate one or a few genes into an adapted or elite variety. The purpose of the study was incorporation into polish wheat germplasm resistance against FHB conditioned by efficient in our climate combination of two resistance loci, i.e. *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A* derived from the Chinese wheat cultivar Sumai3. In addition, increasing grain protein content controlled by *Gpc-B1* locus derived from *Triticum turgidum* var. *dicoccoides*. Selection of BC progeny was focused on target genes using closely linked molecular markers (foreground selection) and recombination events between the target locus and linked flanking markers (recombinant selection). This strategy allowed us to obtain genotypes containing reduced donor chromosome segments with target genes. Preliminary results indicate on elevated resistance to FHB for lines derived using molecular markers in selection process.

Identification and mapping of leaf rust and powdery mildew resistance genes derived from *T. turgidum* ssp. *dicoccum*

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Triticum turgidum ssp. *dicoccum* represents a source of useful genetic variation for several traits including disease resistances. A set of 110 recombinant inbred lines (RILs) was obtained crossing the leaf rust and powdery mildew resistant accession MG5323 of ssp. dicoccum, with the durum wheat cultivar Latino, susceptible to both diseases.

The parents and RIL population were phenotyped, under controlled conditions, with two *Puccinia triticina* (VMC03 and 12766) and one *Blumeria graminis* (O2) isolates.

Marker analysis of the RILs was performed using a large set of molecular markers (SSR, EST-SSR and SNP) leading to the construction of a linkage map containing 10998 polymorphic loci covering the fourteen durum wheat chromosomes with an average genetic distance of 0.62cM.

QTL analysis allowed the identification of one major gene conferring resistance to leaf rust on the short arm of chromosome 1B, explaining a total phenotypic variation ranging from 42.47 to 50.04% and an additional minor gene was located on chromosome 7B, explaining 18.76-25.33% of total phenotypic variation. A single dominant gene for powdery mildew resistance was located on the short arm of chromosome 2B explaining 65.4% of total phenotypic variation.

New resistance genes to leaf rust and powdery mildew in a tetraploid wheat genetic background were identified in this work and the closest linked markers identified can be used for marker-assisted selection (MAS) in leaf rust and powdery mildew resistance breeding.

Location of loci for resistance to *Mycosphaerella graminicola*, plant height and heading date through genome-wide association mapping in wheat

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Septoria leaf blotch, caused by *Mycosphaerella graminicola* (Fuckel) Schrot. (anamorph, *Septoria tritici* Rob. ex Desm) is a major disease of wheat (*Triticum aestivum* L.) worldwide. There are phenotypic studies reporting genetic associations between resistance, plant height and heading date, while others argue that this association is rather due to epidemiological or environmental factors. The aims of this work were to identify (i) marker-trait associations (MTAs) for resistance to M. graminicola, plant height and heading date through genome-wide association mapping DArT-based, (ii) the presence / absence of genetic linkage between those traits. The test material consisted of 96 winter wheat accessions originated from 21 countries genotyped with 874 DArT markers. Three field experiments were conducted at the National University of La Plata, Argentina, during 2012 and 2013 with a split plot design. The entire collection was inoculated with two isolates from two locations in Argentina (Pla and Nueve de Julio) using a concentration of 5×10^6 spores ml^{-1} and sprayed at the 2-leaf stage in both years. For two of the experiments, necrosis percentage in seedlings was scored, whereas for the three experiments, heading date and plant height was evaluated. The percentage of necrosis, plant height and heading date scores indicated a wide phenotypic variation ranging from 32.4% to 67.6%; 22.97 to 127.7 cm.; and 94 to 138 days respectively. Phenotype–genotype association analysis employing the general linear model (GLM) and mixed linear model (MLM) were performed. Only loci significant with both models were considered associated to the traits. QTLs for *M. graminicola* resistance were detected on chromosome 1A (two) and 6B for both experiments with the isolate from Pla. In addition, four significant MTAs on chromosome 1B (two), 2A and 2D for both experiments were effective against the isolate from Nueve de Julio. For heading date, five significant MTAs were detected on chromosomes 1B, 2B, 4B, 5D and 6A for the three experiments. For plant height, four significant MTAs were identified on chromosomes 2B, 3A, 4A and 7A for the three experiments. Necrosis was negatively associated with both plant height and heading date for both isolates, although for one of the isolates it was only significant for heading date. Only the marker WPt6240 was significantly associated with both heading date and resistance to Nueve de Julio isolate considering the significant MTAs for all experiments.

Screening of winter wheat breeding lines for resistance to *Fusarium* head blight and accumulation of *Fusarium* toxins in grain

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Fusarium head blight is an increasing problem in Poland and Europe. This is linked to prevalence of cereal monoculture, reduced tillage and increasing acreage of maize which serve as a source of primary inoculums. Facing this problem, we are making efforts to improve resistance of Polish wheat cultivars to FHB. Cultivar resistance is one of key factors in reducing FHB risk.

We evaluated resistance of advanced breeding lines and check cultivars of winter wheat. Lines were sown in field experiments located in Cerekwica, Western Poland and in Radzików, Central Poland. Wheat heads were inoculated at the flowering stage with *Fusarium culmorum* isolates (DON, NIV chemotypes). Index of Fusarium head blight (FHB) was scored. After the harvest the number of *Fusarium* damaged kernels (FDK) was determined. Fifty one lines combining resistance to *Fusarium* head infection and good agronomic characters and check lines/cultivars (high yielding, resistant, susceptible) were selected. These lines were next examined for accumulation of *Fusarium* toxins. Grain was analysed for the concentration of DON and NIV using gas chromatography technique. Concentration of ZEA was analysed with AgraQuant® ZON test kit.

Average FHB in Radzików was 22.2% and ranged from 4.8 to 56.0% and was similar to that in the field experiments in Cerekwica (average 19.9%, ranging from 3.3 to 48.7%). Proportion of FDK was twice higher in Cerekwica (average 57.5%), ranging from 29.4 to 89,8%, in comparison to the results from Radzików (average FDK% 28.4 %, ranging from 11.5 to 48.4%). Chromatographic analysis revealed presence of DON and NIV in wheat grain. Amount of both mycotoxins in grain from Cerekwica was about 10-times higher (20.3 ppm of DON and 18.2 ppm of NIV) than in Radzików (2.6 ppm of DON and 2.8 ppm of NIV). Zearalenone concentration was also higher in Cerekwica (232 ppb) than in Radzików (31 ppb). It ranged from 0 to 749 ppb in Cerekwica and from 0 to 201 ppb in Radzików.

On the basis of the results obtained in two locations in 2013, 9 lines were selected as resistant combining low head and kernel infection and resistance to accumulation of trichothecenes B and zearalenone.

Development of molecular markers for resistance against the orange wheat blossom midge *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) in winter wheat (*Triticum aestivum*)

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The orange wheat blossom midge *Sitodiplosis mosellana* is a serious pest of wheat in all wheat growing areas of the Northern hemisphere. Although larvae of *S. mosellana* have been reported feeding on various grasses, e.g. barley, rye and wild grass species, economically most important damages are on wheat. Larvae feeding causes shrivelling up to complete destruction of kernels resulting in yield and quality losses. The estimated losses varied from 6-30% (Canada) up to 40 % (Finland). Chemical control with insecticides is difficult, because on the one hand there is no clear correlation between the number of midges caught in pheromone traps and the infestation level of ears and on the other hand actually no forecasting model for population development exists. Therefore, to minimize the losses in yield and quality of wheat and also the negative impact of insecticide treatments, breeding of resistant cultivars is of prime importance. But, the hidden lifestyle of the gall midge larva is hampering an effective selection of resistant genotypes, since manual counting of the number of larvae is very time consuming. Therefore, molecular markers are efficient tools to accelerate the development of resistant varieties.

For marker development two DH–populations, i.e. Robigus(r) x Petrus (s) and Scalmeje(r) x Hermann(s) were phenotypically characterized on 4 locations during three years under natural infestation conditions. Based on this phenotypic data the *Sm1* gene was localized on chromosome 2B with the help of SSR- and DArT-markers. This major QTL explains 60.3 % of the phenotypic variance in the DH-population Scalmeje(r) x Hermann(s) and 71.9% in the population Robigus x Petrus and maybe regarded as a major gene, therefore. Besides this, two additional QTLs were found on chromosomes 4B and 4D each explaining 4% of the phenotypic variance. Two of the DArT-markers linked to *Sm1* were converted in PCR-markers and the analysis of 42 lines carrying the *Sm1* gene revealed that these markers are more diagnostic than the already published WM-1 marker. Therefore, both markers are useful tools for resistance breeding against the wheat blossom midge.

Phenotypic and molecular approach of durable resistance to leaf rust in wheat

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Development of resistance to diseases of rust complex in bread winter wheat continues to be a dynamic and of outmost importance breeding objective at NARDI Fundulea. Leaf rust caused by Puccinia triticina is the most prevalent in wheat areas across the country, but impact of P. triticina f. sp. tritici and P. striiformis f. sp. tritici, could be also hazardous in favorable conditions. To face new challenges such as impact of global climatic change, continuous pathogen evolution, erosion of race specific resistance gene(s), increasing demand of food supply, alternative approaches to resistance management are requested. Advances in understanding the genetic basis of slow-rusting concept, intermediated by discover of functional markers associated with designated race non-specific or adult plant resistance genes (APR) certify that it represents a long-term breeding solution in rust control.

Having in mind the importance of developing and deployment of new wheat cultivars with such resistance, our efforts are focused on: (1)screening Romanian breeding germplasm for the presence of the known slow-rusting resistance genes, Lr34 (the most documented and probably more efficient) and Lr46 and (ii)generating new combinations between resistance genes Lr34, Lr46, Lr67 and modern varieties susceptible to leaf rust.

Preliminary DNA analyses performed with markers STS-csLV34 and cssfr5 evidentiated the presence of Lr34 resistance gene in 21 of 47 cultivars and respectively in 38 of similar number of investigated lines. Scorings of rust resistance expressed as severity (%) and AUDPC, under field artificial inoculation revealed a large variation of all components. Lower values of severity and AUDPC, registered in carriers of Lr34 suggest a more efficient response to leaf rust, but the possible contribution of other resistance genes is not yet fully documented.

Evaluation of derivatives of Lr34/Lr46 cross with specific markers cssfr1(Lr34+) and wmc44 (Lr46+) allows identification of genotypes carrying either one or both of these genes, e.g. line DuRes 116/2013(Lr34+/Lr46+).

Marker assisted selection of slow-rusting resistance to leaf rust, conferred by newly designated resistance genes, combined with desirable agronomic traits will be further approached.

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Identification of resistant genotypes against blast, sheath blight diseases in Western Nepal

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The production and productivity of rice in Nepal is much lower than the neighboring countries. The main factors imparting the rice productivity in Nepal are blast disease, caused by *Pyricularia grisea* and sheath blight caused by *Rhizoctonia solan.* Blast is more problematic in hilly area however sheath blight is in terai mostly with the dwarf and highly tillering varieties. Therefore identification of disease resistant high yielding rice varieties is the main priorities area of rice research in Nepal. A total of 106 promising rice genotypes including susceptible checks were screened against the 2 major diseases of rice (blast and sheath blight) under natural epiphytotic condition at RARS, Khajura, Banke, Nepal in main rice growing season of 2013 (June- October). Each genotype were planted in rod row design with single replication, each of 1.0 m length, 2 rows/genotypes, the rows were kept 10 cm apart and whole nursery were bordered around by 2 lines of susceptible genotypes (Sankara). Disease scoring was done in 0-9 scale to the randomly selected 5 plants from each genotype. Results showed that among the tested 106 genotypes 12 were found to be resistant (HUA 565, WANXIAW763, CT 18493-3-1-1-3VI-3, SACG 4, ZX115, D4098, HUA 564, 53382-2D-KN-4-1, IR82635-B-B-82-2, IR-BB51, IR8) to blast, while the 56 were resistant to sheath blight. Similarly 46 genotypes were moderately resistant, 31 moderately susceptible and 17 highly susceptible with blast while one was moderately resistant, 10 moderately susceptible and 39 were highly susceptible with sheath blight at natural inoculation condition. Thus the identified resistant varieties could be used in advanced rice breeding program for development of resistant and high yielding varieties in Nepal.

Defense gene expression during the development of barley seedlings depends on the level of plastid/nucleus located *WHIRLY1*

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WHIRLY1 is a DNA/RNA binding protein which in barley leaves has been detected in chloroplasts as well as in the nucleus (Grabowski et al. 2008). Due to its dual localization it is an excellent candidate for communication between the two compartments which is required for adaptation of plants to adverse environmental conditions. To investigate its involvement in stress signalling transgenic barley seedlings with an RNAi mediated knockdown of the *WHIRLY1* gene were exposed to continuous high light. The reduction in the level of *WHIRLY1* correlated quantitatively with a severe disturbance in chloroplast development. Impaired chloroplast development coincided with enhanced ratio of xanthophylls/chlorophylls and a higher de-epoxidation state of xanthophyll cycle pigments. During illumination thylakoids prepared from *WHIRLY1* deficient chloroplasts produced more ROS than the thylakoids from control chloroplasts indicating an excess of absorbed light which cannot be used for photosynthesis. In the leaves of high light grown wild type seedlings expression of the defense genes *PR10, PR1 and S40* was dramatically enhanced, whereas the levels of defense gene expression in the *WHIRLY1* deficient leaves was rather low. The results indicate that *WHIRLY1* is required for the establishment of resistance towards excess light. It is proposed that the level of *WHIRLY1* pre-determines resistance of barley towards stress and might be used for selection of barley with enhanced stress resistance.

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Genetic architecture of *Fusarium* head blight resistance in European winter wheat

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Fusarium head blight (FHB) is one of the most severe fungal diseases of wheat and mainly caused by *Fusarium graminearum* and *Fusarium culmorum.* A genome-wide association study (GWAS) for resistance to *Fusarium* head blight was performed using a panel of 358 European winter wheat varieties plus 14 spring wheat varieties. All varieties were grown in the seasons 2008/2009 and 2009/2010 in two locations with three complete replications at each site. Best linear unbiased estimations (BLUEs) were calculated across the four field trials, and the FHB-BLUEs scores ranged from 0.07 (most resistant) to 33.67 (most susceptible). The analysis of marker-trait associations (MTAs) was carried out using a genotypic dataset of 732 microsatellite markers from the publication of Kollers et al. (2013). Meanwhile, the genotyping dataset was extended with 7934 SNP markers derived from the Infinium 90K iSelect-wheat chip. A total of 2497 marker-trait associations were detected by considering associations with a $-\log_{10}$ (P-value) above 3.0 as significant. The results showed consistent associations on all chromosomes. An integrated genetic map of the SSR and SNP markers was constructed to compare the obtained results with the previously reported MTAs for the SSR markers. We will present the analysis of marker-trait associations and the implications for wheat breeding.

Mapping resistance to Argentinean *Fusarium* (*graminearum*) head blight in the ITMI set of RILs of wheat

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Fusarium head blight (FHB) of wheat has become a serious threat to wheat crops in numerous countries with reductions of yield and quality. The current genetic base of resistance to FHB is mainly based on Sumai3 genes worldwide. Argentinean wheats are usually subjected to FHB with losses that range from 10 to 42% of the production. To accelerate the improvement of scab tolerance in wheat, is critical to search for new sources of resistance, since populations of *F. graminearum* are highly heterogeneous. This research was aimed at assessing the ITMI mapping population of 114 RILs for type I and II of resistance against a wide population of Argentinean isolates of *F. graminearum.* Trials were carried out during five years. The severity (S), the Fusarium Index (FI), the 1000 weight grains (TWG) and the Fusarium damaged kernels (FDK) were recorded in three sets of ITMI population subjected to inoculation for type I or II of resistance and in control plants without inoculation. The cultivar Sumai3 was used as a resistant control. There were highly significant differences between both parents of the RILs, with Synthetic showing similar values to Sumai3 for the different traits evaluated. Several of the assessed traits showed significant association to molecular markers of ITMI mapping population. Two major QTLs explained the variability for both types of resistance. Several additional QTLs with effects of lower magnitude have also been identified. The increasing alleles provided by Synthetic contributed to resistance against Argentinean population of *F. graminearum*. These new genes could be incorporated into wheat genotypes already carrying other genes conferring tolerance to FHB that will result in gene pyramiding to control this pathogen.

Involvement of a *PECTINESTERASE INHIBITOR* gene family in mediating resistance to *Rhynchosporium commune* in barley

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The *Rrs2* gene confers resistance to the fungal pathogen *Rhynchosporium commune* which causes leaf scald, a major disease in barley (*Hordeum vulgare* L.). It was fine-mapped to a co-segregating genomic region on the distal part of chromosome 7HS in the cultivar 'Atlas' by Hanemann et al. (2009). Within that region, several genes were identified that belong to the family of *PECTINESTERASE INHIBITOR* (*PEI*) genes whose haplotype differed between a set of analyzed resistant and susceptible barley varieties. Sequence analysis revealed several SNPs within the open reading frame of some family members that lead to amino acid exchanges as well as to insertions or deletions of nucleotides that cause premature stop codons and hence shortened proteins.

Silencing of the complete *PEI* family was carried out by using a VIGS-based (virus-induced gene silencing) assay, targeting regions conserved between the different identified candidates. Knockdown enhanced susceptibility of resistant cultivar 'Atlas' to *R. commune* and enabled the fungus to grow. This effect indicates the involvement of PEIs in the resistance response to the attacking pathogen. In addition, stable transgenic lines were generated that over-express the genes *PEI2*, *PEI3*, *PEI4* or *PEI6* in the background of the susceptible barley variety 'Golden Promise'. These lines were tested for their resistance to *R. commune* by qPCR-based quantification of fungal biomass. However, first results indicate that at least PEI2 alone seems not to be sufficient to confer resistance.

The influence of cold hardening and infection by *Microdochium nivale* on the β-1,3-glucanase and chitinase activities in winter *Triticale (*x *Triticosecale* Wittm.*)*

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The accumulation of pathogenesis-related proteins such as β-1,3-glucanases and chitinases was studied in cold induced snow mould resistance in two Polish cultivars of winter triticale, cv. Hewo and cv. Magnat that substantially differ in resistance to *Microdochium nivale.* The plants were pre-hardened at 12°C for 10 days and hardened at 4°C for 28 days. Subsequently, cold hardened plants were inoculated with fungal mycelium (*M. nivale*) and incubated at 4°C for 7 days in dark. Cold acclimatisation resulted in suppression of the total glucanase and chitinases activities in the resistant Hewo as well as sensitive Magnat cultivars that possibly coincides with altered metabolism. However, upon infection with *M. nivale* the chitinases were markedly induced in the cv. Hewo. At the same time, total β-1,3 glucanases activities did not seem to be affected by fungus in any of the tested triticale cultivars. The pattern and/or the activity of chitinases in plants might be indicative for the resistance/susceptibility against *M. nivale.*

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Identification of new sources of resistance to powdery mildew among *Avena sterilis* L. genotypes

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Powdery mildew caused by *Blumeria graminis* DC. f. sp. *avenae* Em. Marchal. is a deleterious foliar disease of common oat which appears in many parts of the world. Resistance existing in oat cultivars is breaking down by new races of this pathogen and because of this fact there is necessity to look for new and effective sources of resistance to powdery mildew in oat. Previous study showed that valuable sources of resistant could be found in wild relatives of *A. sativa*, especially in genotypes on the same ploidy level. The aim of the present study was identification of new sources of resistance to powdery mildew among *Avena sterilis* L. accessions from different part of the world. The material for this study consisted 50 genotypes belonging to *A. sterilis* species. Host-pathogen tests were used to identify resistance to powdery mildew. As a control, cultivars and line with documented OMR group were used: Bruno (OMR1), Jumbo (OMR2), Mostyn (OMR3), AV1860 (OMR4), and Fuchs - susceptible to powdery mildew. Control plant materials were kindly supplied by Sai L.K. Hsam from the Technical University Munich, Germany.

Presented study confirms that genotypes belonging to *Avena sterilis* species could be used as a valuable sources of resistance to powdery mildew in oat. The majority of analyzed genotypes were resistant to isolate M5 or showed intermediate response. Among tested genotypes 50% were susceptible after inoculation of M11 isolate, the rest of them were resistant or showed susceptible response.

Because of fact that powdery mildew evolve very fast and breaking down resistance determined by single genes, the best whey to obtain resistant cultivars is piramidysation of effective genes or genes which showed intermediate response to powdery mildew. Presented study showed that many *A. sterilis* genotypes should be used in breeding programmes to improved resistance level to powdery mildew in cultivated oat.

Genomics based marker saturation of a BaYMV/BaYMV-2 resistance gene located on barley chromosome 5H

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Soil-borne *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) causing Barley yellow mosaic virus disease are serious threats to winter barley production in Europe and Asia. Due to the transmission by the plasmodiophorid *Polymyxa graminis* chemical measures to ensure barley production in the growing area of infested field are neither effective nor acceptable for ecological reasons. Hence breeding for resistance is the only way to avoid high yield losses. Up to now at least 9 loci conferring resistance to the different strains of BaMMV and BaYMV are known. Recently a new gene was discovered being only effective against BaYMV and BaYMV-2 located on chromosome 5H. In order to identify the structure and function of the gene underlying this resistance a map based cloning approach was carried out. 5085 F_2 plants corresponding to a resolution of 0.0098% recombination derived from the cross 'HHOR4224' x 'Igri' were examined with co-dominant flanking markers to construct a high resolution mapping population. The target interval carrying the resistance locus was estimated at 12.08 % recombination in this population. Marker saturation of this target interval is conducted by using all marker and sequence information available in barley, and employing synteny to rice, sorghum, Brachypodium and newly developed sequence information of barley included in the genome zipper. Until now, 23 additional markers have been mapped in the gene carrying interval. Besides this, all 707 segmental recombinant inbreed lines identified were tested on two locations for BaYMV/BaYMV-2 resistance. The segregation ratio observed revealed a good fit to a segregation ratio of 1r:1s (X^2=0.88). Based on these data and the newly developed markers the BaYMV/BaYMV-2 resistance gene was located between markers 1_xxx1 and Bradi4gxx2 in an interval of 0.41% recombination. Further marker saturation will be conducted using an exome capture sequencing approach.

Xanthomonas translucens – do type III/TAL effectors contribute to pathogenicity?

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Bacterial Leaf Streak (BLS) is the most common bacterial disease of small grain cereals, such as wheat, barley and triticale. *Xanthomonas translucens* (*Xt*) is the bacterial agent responsible of BLS. This disease has been reported at diverse locations worldwide until the end of last century and received increased attention in recent years. BLS of barley and wheat is a seed-borne disease and thus a constraint for international germplasm exchange. Several countries list *Xt* as a quarantine organism. Yield losses as high as 40 percent have occurred in the most severely diseased fields in the United States, although losses are generally 10 percent or less. To cause disease, most xanthomonads depend on a highly conserved type III secretion system which translocates type III effectors (T3Es) into target host cells. A specific family of T3Es, called Transcription Activator-Like (TAL) effectors, modulate plant gene expression upon specific binding to target boxes in the promoter regions of plant genes. Recognition is due to a central region of the TAL effector protein consisting of nearly identical repeats with two hypervariable residues in each repeat specifically recognizing one DNA base pair of the target box. By mimicking eukaryotic transcription factors, TAL effectors induce specific plant gene(s) whose activities are required to establish a susceptible state of the host towards infection. Virtually nothing is known about pathogenicity mechanisms of *Xt*. Previous work and several new draft genome sequences have proven the presence of TAL effectors in *Xt*. Our objective is to find key virulence determinants involved in the interaction between cereals and *Xt*. Several type III secretion *hrc* genes and *tal* genes were knocked out and mutants were characterized by pathogenicity assays. In parallel, *tal* genes were cloned and sequenced in order to identify candidate susceptibility genes in barley.

Modelling effector-receptor interactions and susceptibility to *Septoria nodorum* blotch in wheat

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Fungal effector-host sensitivity gene interactions play a key role in determining the outcome of septoria nodorum blotch (SNB) on wheat. These interactions are quantitative and involve the interaction of multiple effector-sensitivity gene systems. Three effectors are well characterised in this pathosystem; SnToxA, SnTox1 and SnTox3. We used a double haploid (DH) population derived from the wheat cultivars Calingiri and Wyalkatchem (CxW) to study the inheritance of sensitivity to SnTox1 and SnTox3 (neither parent is sensitive to SnToxA) and of disease susceptibility. SnTox1 recognition is encoded by Snn1 and SnTox3 recognition is encoded by Snn3. Interval QTL mapping showed that SnTox1-Snn1 interaction was involved in the response in the development of SNB on seedlings as well as adult plants using various evaluation indicators. Surprisingly, no effect of the SnTox3-Snn3 interaction was observed. Gene expression analysis showed that SnTox1 expression was induced by Snn1 but that SnTox3 expression was not induced under any circumstances. We suggest that differential expression of effectors explains the relative impact of Snn1 and Snn3 on the outcome of the disease.

This study also identified another QTL, on 4BL, was associated with response of the CxW population to semi-purified Tox3 and QTLs on 2AS, 3A and 6B were only detected at SNB seedling in greenhouse and adult field trial, respectively. Outcomes from this study confirmed that sensitivity gene Snn1 responding to SnTox1 effector appear as the main SNB contributor on the CxW DH mapping population from seedling to adult plants in different environments and explained the dominance of SnTox1-Snn1 over SnTox3-Snn3 interactions.

Allelic variation at powdery mildew resistance gene *Pm3* in a collection of tetraploid wheats

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Powdery mildew, caused by the biotrophic fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a prevalent and devastating wheat disease occurring world-wide in temperate climates. Race-specific resistance to wheat powdery mildew is controlled by the *Pm* genes. *Pm3*, the one resistance gene cloned, is localized on the short arm of wheat chromosome 1A and is present in 17 functional allelic forms (*Pm3a* to *Pm3g, Pm3k* to *Pm3t).* The search for new *Pm3* alleles was carried out on a collection of 233 tetraploid wheat genotypes (*Triticum turgidum* L.), grown in the experimental field of the University of Bari. This screening led to the identification of 34 accessions (14,6%) resistant to the isolate O2 (virulence/avirulence pattern: *Pm1, Pm2, Pm3, Pm4a, Pm4b, Pm5, Pm6, Mli / Pm3a, Pm3b, Pm3d).*

Out of 233 lines a total of 129 lines (55.4%), both resistant and susceptible, amplified a *Pm3* gene: 122 showed a fragment of 1,200 bp, 5 lines a fragment of 946 bp and 2 lines led both fragments. The 946 bp fragment was localized on the chromosome 1A using nulli-tetrasomic lines of Chinese Spring.

The two fragments were sequenced and the comparison between the 946 bp fragment with the known alleles *Pm3a, Pm3b, Pm3d* showed an overall similarity, while the 1,200 bp fragment exhibited 3 insertions of 47 bp, 17 bp and 8 bp, respectively. All the lines were screened with *Pm3* allele specific primer combinations, but a positive PCR reaction was observed only for the *Pm3b* allele. Eight of these lines, four resistant and four susceptible, were further analyzed with a set of PCR primer combination covering all the *Pm3b* coding region sequence. Out of eight lines analyzed, the line AG-85 of ssp. *durum*, showed the higher level of polymorphism compared to Chinese Spring *Pm3b* gene sequences. Then, the coding region was aligned against all *Pm3b* gene–like sequences available in public database. Several differences in terms of SNPs and small indels were found, resulting in new allelic variants of the *Pm3b* gene.

Relationship between *Fusarium* head blight resistance and yield characteristics in a bread wheat mapping population

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Breeding of high-yielding quality wheat with improved resistance to *Fusarium* head blight (FHB) is a big challenge in most wheat producing countries. Difficulties result from the complexity of FHB resistance, its quantitative inheritance, and the genetic linkage of resistance QTLs (quantitative trait loci) and chromosome regions affecting important bread wheat characteristics.

A mapping population was produced from the cross of Ning8331 and Martonvásári 17. Alleles of wheat lines were analysed with microsatellite and AFLP assays.

Construction of linkage map resulted in 44 linkage groups. Out of these, 29 linkage groups were identified having significant QTL effect on examined characteristics.

QTLs associated with FHB resistance which showed no linkage to QTLs for yield components usually had little effect on FHB severity. Three chromosome regions influenced the field resistance remarkably (evaluated by spike infection, kernel infection and yield reduction), and all of them had a significant effect on the yield related traits. The chromosome arm 3BS of Ning8331, in addition to its outstanding effect on either field or type II resistance, played an important role in decreasing of thousand kernel weight (TKW) and kernel weight per spike (KW). A 5A chromosome region, influencing spike infection, determined both test weight and KW primarily. The chromosome 4B of Ning8331 reduced kernel infection and had a positive effect on TKW. However, it decreased the kernel number and caused significant increase in plant height.

The most effective FHB resistance QTL was identified using single spikelet inoculation, and was found to be located on the chromosome arm 2DL. This locus has an increased importance, since it has no linkage with any yield and phenotypic characteristics examined in our study. Therefore, this QTL may have greater potential in the breeding of high-yielding wheat cultivars with good FHB resistance.

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Genetic mapping of stem, leaf and stripe rust resistance in a spring wheat population using a 90K SNP Infinium iSelect assay

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Three wheat rusts [stripe rust (caused by *Puccinia striiformis Westend* f. sp. *Tritici*), leaf rust (caused by *Puccinia triticina* Eriks), and stem rust (caused by *Puccinia graminis* f. sp. *Tritici*)] are devastating diseases that can cause substantial economic yield loss in western Canada. Identification of novel sources of resistance to these diseases and their introgression into wheat cultivars through marker assisted selection (MAS) is a priority in many breeding programs. The spring wheat germplasm line N9195 expresses resistance to the prevalent races of all three rusts in western Canada. To understand the genetics of resistance in N9195, a doubled haploid mapping population was generated from an AC Reed/N9195 cross, where AC Reed is susceptible to all three rusts. This mapping population was phenotyped for stem, leaf and stripe rust in the field as well as genotyped using a 90K SNP Infinium iSelect assay. A high density genetic linkage map (total length 3035.78 cM) for the population was constructed using 3808 high-quality SNP markers belonging to 25 linkage groups spanning 20 wheat chromosomes (except 3D). The genetic mapping of the rust resistance genes is in progress and these results of will be presented.

Allelic diversity of the *HvGER4* gene cluster and its role in host defence against the barley powdery mildew fungus

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Plants can recognize attacking micro-organisms by perceiving pathogen-associated molecular patterns (PAMPs). The PAMP triggered immunity (PTI) is the underlying mechanism of quantitative host- and of nonhost resistance. The powdery mildew disease caused by the fungus *Blumeria graminis* f sp. *hordei* (*Bgh*) serves as a model for studying PTI in barley. Pathogen-induced genes of barley germin-like protein 4 (*HvGER4*) were found to be PTI components against *Bgh*. The encoded proteins with superoxide-dismutase activity are proposed to be targeted to the plant cell wall at the site of attempted penetration where they may catalyze production of H_2O_2, which has been shown to be a signaling molecule for a range of defense reactions, including cell death, and as a cofactor for cell wall reinforcement by cross-linking. A tandem cluster of eight paralogous genes of *HvGER4* were identified in the barley variety Morex on a physical genome fragment near the distal end of chromosome 4HL.

In frame of the project “BARLEY-fortress” 52 land races of spring barley genotypes plus two cultivars and one wild barley introgression line carrying QTL to *Bgh* resistance were tested for *HvGER4* expression levels by quantitative RT-PCR analysis. These genotypes were divided in 6 different groups: early resistant, late resistant, and susceptible, each of them subdivided to high and low *HvGER4* expressing. Eight candidate genotypes from these groups were chosen to construct BAC libraries for further analysis after confirming the *HvGER4* expression level in a northern blot analysis. The BAC libraries were prepared and screened in cooperation with INRA-CNRGV in Toulouse, France. Some of the positively screened BAC clones were sequenced by Next Generation Sequencing (NGS) and all but one were confirmed to contain germin-like sequences. Further positive BAC clones will be sequenced in order study the nature, abundance and the organization of the *HvGER4* genes in different genotypes and its role in the interaction with *Bgh*.

In a parallel approach 37 transgenic RNAi T1 events putatively silenced for *HvGER4* genes were phenotyped regarding resistance to *Bgh* and GER4 expression, and 9 lines were selected for producing T2 and homozygous T3 generation.

Molecular-cytogenetic identification of rust resistance genes in Russian wheat hybrids and varieties and their use in marker-assisted breeding

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Wheat hybrids and varieties carrying translocations from related species or genera are an important source of rust resistance genes. Here we characterized Russian wheat varieties and hybrid lines (*Triticum aestivum/T. timopheevii, T. aestivum/Ae. speltoides*) using C-banding, *in situ* hybridization and SSR analysis.

It was shown that 1RS.1BL translocation is most common (5 of 25 varieties analyzed). Some varieties carry other types of introgressions that have not been identified yet, namely 2DS.2SL, 5BS.5BL-5GL, and 6BS.6BL-6GL translocations, 6D/6Agi substitution from *Agropyron intermedium* and introgression of *Aegilops tauschii* genetic material into 1D and 6D chromosomes. The chromosome 6D/6Agi of cultivar Tulaikovskaya possesses genes conferring resistance to leaf, stem, and yellow rusts and powdery mildew, which are not allelic to any known rust resistance genes. On the basis of EST analysis we selected markers specific for 6Agi chromosome for their subsequent use in molecular-assisted breeding.

Four lines with durable resistance to leaf rust were selected from 74 hybrid lines. Marker-assisted backcrossing was used for developing lines carrying a single *Ae. speltoides* and *T. timopheevii* translocations. It was shown that lines carrying the translocations from *Ae. speltoides* (21-4 with T5BS.5BL-5SL, 17-7 with T6BS.6BL-6SL) are resistant to leaf rust. The line11-8 with 7D/7S substitution is completely resistant to leaf rust and powdery mildew. The lines 5366-180 and 3862-5 with *T. timopheevii* translocations were resistant to leaf rust and stem rust, respectively. According to molecular analysis line 3862-5 may possess the *Sr36* gene. The *LrTt2* gene of 5366-180 may be allelic to *Lr18*. The rust resistance genes identified in other selected lines (21-4, 17-7, 11-8) haven't been described earlier.

The variety Tulaikovskaya and lines 21-4, 17-7, 11-8, 3862-5, 5366-180 were used as donors of resistance genes in molecular-assisted breeding of spring and winter wheat cultivars. As a result the spring wheat lines with high productivity and resistance to fungal diseases have been developed. The development of winter wheat lines are being in progress.

High-resolution analysis of a locus for resistance to wheat *Stagonospora nodorum* glume blotch and evaluation of the impact of plant morphology in the disease resistance

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Stagonospora nodorum glume blotch (SNG), is a necrotophic fungal disease affecting spikes in bread wheat (*Triticum aestivum* L.) mostly under humid conditions and mild temperatures. Such climatic conditions are quite typical in many areas used for wheat production, particularly during the grain filling period. Thus, this pathogen can result in devastating disease and yield losses of up to 30-40%, affecting also grain quality. Resistance to SNG is known to be quantitative and classical genetic analysis has revealed several QTLs contributing to the resistance. However, very little is known at the molecular and physiological level on this resistance.

Our ultimate aim is the high-resolution mapping of the wheat resistance QTL against SNG previously genetically characterized as a QTL (QSng.sfr-3BS) in chromosome 3B. To accomplish this, a high resolution genetic map for the QTL target region was constructed. The combined analysis from the field evaluation of the mapping population of near-isogenic lines (NIL) and genotyping data revealed that two linked loci are associated with SNG resistance, one of them also involved in plant height.

In line with this last aspect, we tested if any other morphological traits might be correlated with SNG resistance. Previous work focused on the resistance against diseases affecting cereal spikes revealed a non-negligible influence of spike morphology, peduncle length and epicuticular wax content on resistance. More generally, genotypes with short peduncles and compact spikes have a faster disease spread with higher infection rates than genotypes with long peduncles and a lax spike. With the aim to test if the "spike environment" has an active role in the resistance, the peduncle length, spike density and mean distance between the spikeletes were measured in our NIL population across three contrasting environments. In agreement with previous findings, we observed that genotypes with shorter peduncles and more compact spikes were more susceptible to the disease, probably creating a more humid environment and allowing an easier spread of the pathogen. Also, those spikes with a higher epicuticular wax content showed lower rates of disease infection. The degree of association between all these plant-morphological characters and SNG resistance is discussed.

Characterization of the prehaustorial resistance against leaf rust (*Puccinia triticina* f. sp. *tritici*) in Einkorn (*Triticum monococcum)* by massive analysis of cDNA ends (MACE)

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Leaf rust caused by *Puccinia triticina* f. sp. *tritici* is the most common rust disease of wheat and causes high yield losses worldwide. *Triticum monococcum* accessions are valuable sources for improving leaf rust resistance in hexaploid wheat. In extensive screening programs *T. monococcum* accession *Pi272560* has been identified showing prehaustorial resistance against leaf rust. First experiments revealed that the effective defense reaction is associated with an increased activity of peroxidases and pathogenesis related genes. This race non-specific (horizontal) prehaustorial resistance prevents the infection prior to the formation of haustorial mother cells. Hence the goals of our studies are (i) to analyze the biochemical background of this resistance by microscopy and measurement of the H_2O_2 content in leaves and (ii) to determine the molecular background by genome wide expression studies using the massive analysis of cDNA ends (MACE). A xylenol orange assay revealed higher amounts of H_2O_2 up to 1,88 µM g^{-1} in inoculated leaves 12 to 48 hours after inoculation (hai) in the resistant accession. In order to analyze the expression of genes which led to observed defense reactions, MACE from RNA samples which were isolated within the first 24 hai from resistant and susceptible *T. monococcum* accession has been conducted. Within the time segment from 0-8 hai 120950 tags, between 8-16 hai 95147 tags, and between 16-24 hai 90150 tags could be annotated to the Swissprot database. Using the counts per million determined tags (cpm) up to 8 hai 423, between 8-16 hai 523, and between 16-24 hai 552 tags differentially expressed were identified. These tags have been blasted to the NCBI database and differentially expressed peroxidases (between 9-31), chitinases (2-13), kinases (32-80) and pathogenesis related genes (0-18) were determined. The results show that higher or exclusive expression of peroxidases, chitinases and pathogenesis related genes are involved in prehaustorial resistance. In addition seven genes related to (leaf rust) resistance genes were detected. Based on the analysis of 1136 tags comprising 4358 SNPs, 362 differentially expressed SNP containing genes were mapped *in silico* using the Genomezipper.

Tolerance to foliar diseases in wheat cultivars

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Tan spot (*Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis*) (Died.) Shoem), Septoria leaf blotch (*Septoria tritici* Rob. ex Desm., teleomorph *Mycosphaerella graminicola* (Fuckel) Schrot. in Cohn) and leaf rust (*Puccinia triticina*) are important diseases in wheat worldwide. Disease tolerance is the genotypic ability to maintain yield performance in the presence of disease symptoms. The aim of this work was to evaluate tolerance in 10 wheat cultivars determining if they have a similar tolerance level when they are affected by pathogens with a different nutritional habit and identify possible associations between grain number, wheat quality and components of biomass generation with tolerance. Split-split plots experiments with three replications were conducted, one of them with *P. triticina* (biotrophic) and *P. tritici-repentis* (necrotrophic) inoculations and the other with *S. tritici* (hemibiotrophic) inoculations. Pathogens were the main plot, three inoculation treatments (without inoculation, with low inoculum concentration and with high inoculum concentration) were the subplots, and 10 cultivars were the sub-sub-subplots. Subplots without inoculum were treated with fungicide Orquesta™ Ultra to minimize the presence of natural inoculums. Disease severity, yield components and variables generating biomass and wheat quality were measured. Tolerance was evaluated as the slope of the regression lines between area under disease progress curve and yield for each inoculum treatment, within each cultivar. Treatments inoculated with *P. triticina* caused a higher reduction in accumulated intercepted radiation photosynthetically active compared with treatments inoculated with *P. tritici-repentis*, whereas radiation use efficiency was 35.6% lower in treatments affected by leaf rust compared with those affected by tan spot. Differences in tolerance among cultivars were detected for the three pathogens. For experiments inoculated with *P. triticina* and *P. tritici-repentis*, the interaction pathogen × cultivar was not significant, indicating that tolerance can be effective even with pathogens with a different nutritional habit. Cultivars with a higher grain number did not show higher reductions in kernel weight.There was a slight relationship, explaining 30% and 24% of the variation between accumulated intercepted radiation during grain filling and reduction in kernel size due to tan spot and leaf rust respectively, indicating some source limitation. Some associations were also found between sensitivity (intolerance) and cultivars with the best wheat quality.

Population structure and location of genes for resistance to *Mycosphaerella graminicola*: Recent advances in Argentina

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Leaf blotch of wheat [*Septoria tritici* Rob. ex Desm., teleomorph *Mycosphaerella graminicola* (Fückel) Schröt. in Cohn] causes significant losses in wheat. Results about genetic variability of the pathogen and location of the resistance to the disease, carried out in Argentina are presented. A study with 126 isolates from several locations revealed the existence of 81 different haplotypes, indicating a high degree of genotypic diversity. Furthermore, a high gene flow was found between subregions without significant genetic differences between populations. Virulence tests were conducted on nine selected Argentinean wheat cultivars and 14 foreign cultivars with some level of resistance to the pathogen inoculated with 16 different isolates molecularly characterized in the previous work in two environments. Cultivars with good levels of partial and complete resistance to some isolates were detected in seedlings and the adult stage. During the last decade, 18 *Stb* major genes conferring resistance to the pathogen and several QTL have been identified around the world. Our group determined that chromosome 7D of "Synthetic 6x" had a major resistance QTL against the two isolates tested in seedlings and the adult stage which mapped to the centromeric region (marker *Xgwm44*) of the 7D chromosome and it is likely that the gene involved was *Stb5*, which proved to be effective against isolates originating from both Europe and South America. Major gene effects were also found on chromosomes 5A and 5D of "Synthetic 6x". In addition, a source of resistance has been mapped on chromosome 7D of spelt wheat. Two regions of the chromosome were associated with isolate-specific QTL, one expressed at the seedling in the centromeric region of chromosome 7D (*QStb.ipk-7D1*) which corresponded to the location of the major resistance gene *Stb4* originated from bread wheat cultivar "Tadinia" and *Stb5* originated from *Triticum tauschii.* and another at the adult plant stage on the short arm of chromosome 7D (*QStb.ipk-7D2*) in a similar position to the locus *Lr34/Yr18*, known to be effective against multiple pathogens. Furthermore, using an ITMI mapping population (W7984 × Opata 85), three loci were discovered on the short arms of chromosomes 1D, 2D and 6B at the seedling stage effective to two isolates. At the adult plant stage, two isolate-specific QTL were found at the long arms of chromosomes 3D and 7B.

Dissection of a durable blast resistance in rice trough mapping and RNA-Seq approaches

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Gigante Vercelli (GV) and Vialone Nano (VN) are two old temperate japonica Italian rice cultivars with contrasting response to blast (Magnaporthe oryzae) infection. GV displays a durable and broad resistance, whereas VN is highly susceptible. A GV x VN segregating population, used to develop an SSR-based genetic map, led to the identification of two loci, localized to the long arm of chromosomes 1 and 4, responsible for blast resistance in GV. RNA-Seq was then employed to dissect the early molecular processes deployed during the resistance response of GV and VN at 24 h after blast inoculation. Differential gene expression analysis identified 726 and 699 up regulated genes in response to infection in GV and VN, respectively. GV exhibited a dramatic up-regulation of defense genes and genes involved in the early steps of defence perception-signalling (e.g., MAPK cascades and WRKY transcription factors). Sequences carrying the NBS-LRR domain, a distinguish feature of a major class of plant disease resistance genes, and displaying expression patterns consistent with a potential role as GV-specific functional resistance gene(s) were identified and localized on the Nipponbare reference genome. A co-localization of three of them was observed with the two loci responsible for blast resistance in GV. The integration of genetics and transcriptomics studies has therefore provided candidates for the GV durable resistance. The ongoing de-novo assembly of the GV genome will allow a better definition of the positional relationships between the candidates and the blast resistance loci.

Development of novel, broad *Fusarium* resistances in wheat to meet the special challenges of current bio-energy crop rotations

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In Germany, an important aim of the Renewable Energy Sources Act ("EEG") is the marked increase of biofuel as a petrol substitute by local plant production. For a balanced bioenergy crop rotation, wheat is an interesting biofuel crop since the high-protein stillage is an economically and ecologically important by-product. However, this double usage of wheat is seriously threatened by the *Fusarium* diseases like head blight (FHB), crown and root rot (FCR and FRR), particularly caused by *F. graminearum* infection. Disease outbreaks lead to serious reductions in bioethanol yield and quality of the stillage. As a consequence of current bioenergy crop rotations and changing climate conditions, *Fusarium* diseases are expected to gain significance in the future.

Breeding of resistant wheat cultivars is seen as the most effective management strategy, but still hampered by insufficient knowledge on the complex trait *Fusarium* resistance. Large-scale genetic studies and germplasm screenings are required to bridge existing knowledge gaps, particularly in the field of soil-borne *Fusarium* disease. Therefore, the presented project aims at a global genetic resistance study to assay genome-wide associations for plant responses to both the floral (FHB) and the soil-borne (FRR/FCR) diseases.

A diverse and unique set of 460 wheat accessions has been collected from the worldwide most important *Fusarium* hotspot regions with a potentially diverse and novel resistance spectrum. The Genomic Selection (GS) approach will be applied to select *Fusarium*-resistance donors for further steps in resistance breeding programs. Genotypic data are generated by applying the novel 90K SNP-array that densely covers the whole wheat genome. The applied phenotypic data comprise multi-location FHB field trials as well as improved phenotyping in a reduced training population (n=120 accessions) by using customised FHB/FRR/FCR bioassays established at the IFZ Giessen.

To reveal existing kinships of the accessions, the population structure and genetic diversity of the wheat collection have been analysed based on different statistical computing methods implemented in the *R* software. Knowledge on the degree of relationship in a diverse plant population is a crucial prerequisite for both association studies and GS approaches to avoid false positive findings by identifying spurious marker-trait-associations.

Insights into the *Fusarium*-wheat root pathosystem uncover a hidden danger to wheat production

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Fusarium diseases, particularly caused by *F. graminearum* belong to the most destructive diseases in cereal crops worldwide. These pathogens can basically attack any part of a plant, thereby causing the floral disease *Fusarium* head blight (FHB), and the soil-borne diseases *Fusarium* root rot (FRR) diseases and crown rot (FCR). Generally, *Fusarium* diseases challenge food security by causing significant seed yield reductions and mycotoxin contaminations which seriously compromising seed and flour quality. Particularly the FRR/FCR diseases profit from global warming and climate change. Since the specific characteristics of soil-borne diseases make classical plant protection ineffective, growing resistant varieties is the most promising strategy which, however, is still hampered by limited knowledge on the epidemiology, pathogenesis, and genetics of wheat resistance.

Our studies on the hitherto unknown *F. graminearum*-wheat root pathosystem have demonstrated the high capability of the pathogen to infect and colonize wheat roots at the seedling as well as adult plant stage. Serious impacts on root and seedling/plant development were observed, followed by the invasion and mycotoxin contamination of the entire plant shoot. Even in the heads of FHB and mycotoxin resistant genotypes high toxin loads were measured already at the end of flowering (at least 30-times higher compared to floral infections), which could be a direct consequence of the presence of fungal hyphae or an indirect result of systemic transmission via the colonized vascular stem tissues.

Nevertheless, studies on the FRR disease progress demonstrated variability among the wheat responses ranging from partial resistance to high susceptibility. Histopathological studies in partially resistant and fully susceptible genotypes have uncovered important first indications on the mode of partial resistance in root and hypocotyl tissues, besides plant tissue-specific pathogen-wheat interactions. Major FHB resistances failed to protect against FRR which brings along the high risk that new FHB resistant elite cultivars are insufficient to prevent FRR. However, important commonalities between FHB and FRR were also found which concern the course of diseases as well as the induction of *Fusarium* resistance gene candidates in head and root tissues. Finally, indications for a present Adult Plant Resistance (APR) against *Fusarium* were found which might explain reported differences in the genetics of wheat resistance to floral and soil-borne diseases.

Screening of *Triticale* winter breeding lines for resistance to *Fusarium* head blight and accumulation of *Fusarium* toxins in grain

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Fusarium head blight is a disease of cereals caused by fungi of the *Fusarium* genus. These fungi produce toxic metabolites - mycotoxins. Head infection by *Fusarium* leads to kernel infection and accumulation of mycotoxins in grain.

Resistance to Fusarium head blight of 36 winter triticale lines combining resistance to *Fusarium* head infection and good agronomic characters and check cultivars (high yielding, resistant, susceptible) was tested. Triticale was sown in field experiments in two locations. At flowering, triticale heads were inoculated with three *Fusarium culmorum* isolates. FHB index was scored and after the harvest percentage of *Fusarium* damaged kernels was assessed. Grain was analyzed for a content of trichothecenes B (deoxynivalenol and derivatives, nivalenol), and zearalenone using gas chromatography technique (trichothecenes) and AgraQuant® ZON test kit (zearalenone).

The average FHB indexes were similar in both locations and amounted 10.1% in Radzików, and 7.7%. in Cerekwica. Percentage of *Fusarium* damaged kernels was slightly higher in Cerekwica (43,1 %) than in Radzików (41.8%). The average content of DON in Radzików amounted to 2,242 ppm and was lower than in the second location – 6.607 ppm. In Cerekwica there were also large quantities of NIV in grain. The average content was 13.638 ppm, while in Radzików it was very low – 0.694 ppm. Considerable amounts of DON derivatives in Cerekwica were detected (0.205 ppm of 3AcDON and 0.208 ppm of 15AcDON) and only traces in Radzików. The content of the zearalenone in the grain from Cerekwica was high and amounted to 201 ppb, while in Radzików was five times lower – 37 ppb. Relationships between FHB index and mycotoxin contents were statistically insignificant in both locations, except for ZEA in Cerekwica. FDK percentages correlated significantly with concentration of all *Fusarium* toxins in Cerekwica but not in Radzikow.

On the basis of the results obtained in two locations in 2013, 17 lines were selected as resistant, combining low head and kernel infection and resistance to accumulation of trichothecenes B and zearalenone.

Session V

Quality for Food and Industrial Use

Significance of β-glucan in barley and oat grain for brewing and nutritional breeding

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Within the breeding nurseries of Kazakhstan, the β-glucan content varies from 2.6 to 6.2% in spring and from 2.2 to 5.7% in winter barley grain, increasing under rainfed conditions. For brewing purposes, accessions with a value of this index lower than 4%, some of which displayed relative stability across reseedings, are singled out.

Seven samples of the spring barley were characterized by the β-glucan values less than 4.0% from 3,66 to 3,98% and 4 winter barley samples YK-43, №28.350, 7-AI and 4-AI (3,65-4,00).

Of course, such genotypes of barley produced in the feed and food purposes for which, on the contrary, it is important to the high content of β-glucan. The β-glucan in the grain more than 5.0% for 13-47% formed genotypes, respectively, the region conditions and year of reproduction. Maximum values of β-glucan content in breeding nurseries differed cvs Bereke 54, genotypes K-18, AI- 2, AI-20. d.698. ER Art 1CARDA. Mogos 9-75; wild barley H.spontaneum 705 (Almaty, Kazakhstan) from irrigation samples were isolated WB - 425 and WB -417 as exceeding 5.0 % value content β - glucan (5.41 and 5.66 respectively).

The maximum β-glucan content in the KazRIAPG control nursery the varieties-standards Skakun (5.1%), № 770 (5.0%) and 24/48 (4.9%) were allocated. Among the collection samples by a maximum value were characterized the numbers as K-11247, K-13587, K-13544, K-14638, K-14638, K-14836 from Vavilov IPP-collection.

It was found a correlation between the content of β-glucan and protein 0,60-0,87, the negative conjugation with starch content was determined from r = -0.48 to r = -0.57 and the positive correlation with the grain hardness index (r = 0.59).

The samples researching from the world collection (Sank-Peterbourg) from Aktobe and Almaty reproductions allowed to differentiate its on the β-glucan content: with the content of β-glucan to 30 mg/kg found 44% of genotypes, 30-40 mg/kg - 52% of genotypes and 4% - with a high β-glucan content >50 mg / kg (for the sample K- 14638 in the two reproductions the high content was observed). Selected samples identified by protein and DNA markers.

BREVIARISTATUM-e (*ari-e*), a homolog of the rice heterotrimeric G protein γ subunit DEP1, controls plant height and grain length in barley

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Two loci important for yield in rice, *GS3* and *DEP1*, have been identified as heterotrimeric G protein γ subunits. For the *GS3* locus it was shown that a knockout of the gene results in longer grains, while plants encoding a truncated version of the *GS3* protein produce very short grains. In the case of *DEP1* it was reported that lines encoding a truncated protein show increased panicle branching, denser panicles, shorter grains and reduced plant height.

Barley possesses one gene encoding a GS3/DEP1 type of G protein γ subunit, *HvDEP1*, possibly a homologue of *OsDEP1*. Originally we intended to generate transgenic *HvDEP1* knock out lines, as well as lines overexpressing a truncated *HvDEP1* gene in barley. As Golden Promise is the only cultivar routinely used for barley transformation, we subsequently sequenced *HvDEP1* in Golden Promise prior to transformation studies. We found the gene to carry a single nucleotide insertion in exon 2, leading to a frame shift resulting in a premature stop. As Golden Promise is known to carry the semi-dwarfing gene *ari-e*, we sequenced *HvDEP1* in nine induced *ari-e* mutant lines and found *HvDEP1* mutated or deleted, suggesting that *HvDEP1* is *ari-e*, the semi-dwarfing gene in Golden Promise.

Previously we mapped a major plant height QTL at the *ari-e* locus using a RIL population which was derived from a Golden Promise x Morex (GPMx) cross. Here we confirmed that the *HvDEP1* position coincides with the plant height QTL in the GPMx population. Additionally, we measured and mapped spike and grain size parameters, demonstrating that the grain length, but not width, QTL peak coincides with the *HvDEP1* position. The *ari-e* locus does not affect tillering in barley, which is consistent with findings in rice.

Kernel size distribution, yield parameters and quality of Latvian grown oat cultivars

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Oat (*Avena sativa* L.) is recognised as healthy food in the world. As raw material for food it contains significant amounts of healthy substances as vitamin E and β-glucan. The task of the study was to characterize 5 husked and 2 naked oat cultivars in their biochemical structure, yield and parameters of productivity as well as grain size, which is a very important feature for food producers. Experiments were carried out at State Stende Cereals Breading Institute in the years 2011, 2012 and 2013. To obtain an equal research background all cultivars were grown in a plant breeding crop rotation field, with similar growing conditions (sowing-time, fertilizer, plant protection activities), which agree with generally accepted technology of oat cultivation in Latvia. Experiments were done in four replication n randomized block design. Biochemical parameters were tested by using Infratec Analyser 1241. ANOVA procedures were used for data analysis. Yield significantly higher were observed for husked oat cultivars. Significant differences were in lipid content showing higher amount for naked oat cultivars. Naked oat breeding line 'S-156' showed the highest β-glucan content in both years. Results of kernel size measurements showed that significantly larger grains were observed for variety 'Scorpion' – nearly 80% of kernels were larger than 2.2 mm in diameter what would be good for food producers, but quality parameters of this variety were average. These results could be used as a promising material in the breeding work of naked and husked oat varieties.

Antioxidant capacities of rye flour and five experimental bread model systems: Comparison between genetic and technological factors

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In the rye grain, dietary phenolics occur mainly as insoluble components. The antioxidant properties of rye-based products are related to their bioavailability, which may be improved by processing. The antioxidant activities of rye flours and five experimental bread model systems were investigated in both aqueous and lipid phases. The bread model systems were produced by the direct bread-making procedures without (A) and with lactic acid addition (B) as well as three-stage sourdough methods (C, D and E) with 12-, 24- and 48-h fermentation of first stage, the native starter preparations. The total antioxidant capacity determined as Trolox equivalent (TEAC) in PBS extract of whole-meal flours from five rye cultivars was almost two times higher than that found in their methanolic extracts. Only bread B, obtained by direct lactic acid addition, showed higher or comparable TEAC value, in comparison to that of starting flour, depending on the rye cultivar used. While, a significant decrease in these values were observed in the remaining breads, however, the differences between bread A and sourdough breads C, D and E were specific for an individual rye cultivar. In methanolic extracts, the TEAC values of all types of rye bread were lower than those of corresponding flours. These decreases were influenced by genetic factors, but there were small differences between the TEAC values across different types of bread obtained for each rye cultivar, excluding bread B with slightly lower values. The total antioxidant capacity of rye breads in lipid phase was significantly correlated with the level of free phenolic acids. Also, it was associated with conjugated and ester-linked phenolic acids present in the sourdough breads. Whereas, the TEAC values found in the PBS extracts were poorly correlated with different classes of phenolic acids. In the next stage of this study, the flour and bread samples will be screened for other phytochemicals and other *in vitro* assays will be applied to find out how to maximise the antioxidant potential of rye bread.

Molecular characterization of *Glu-B3* locus in segregating populations and varieties

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The role of low molecular weight glutenins (LMW-GS) in bread wheat (*Triticum aestivum* L.) functional quality is not well understood yet, mainly due to their genetic complexity and the lack of efficient methods to distinguish among members of this multigene family. Additionally, the use of different nomenclatures and standards for LMW-GS assignment by different groups makes it difficult to disclose genotype-quality relationships. The development of molecular markers for genetic characterization and allele determination of LMW-GS is demanded. Nowadays, the most promising marker-based system combines the analysis of the complete gene family by PCR amplification with a pool of primers designed in conserved regions of LMW-GS genes and high resolution capillary electrophoresis (CE). This method has been developed in varieties but had never been tested in segregating populations. In this work, DNA from 95 F_2 individuals and 65 lines of a $F_{4:6}$ population, both derived from a 'Tigre' x 'Gazul' cross, was amplified and analyzed by CE. Sixteen polymorphic fragments, belonging to the *Glu-B3* locus, were scored as present (1) or absent (0). The mendelian segregation pattern observed in both samples validated the suitability of this system in the characterization of LMW-GS in segregating populations for assessing genotype influence on quality. One pair of *Glu-B3* alternative fragments significantly influenced alveograph quality parameters: lines with the 'Tigre' fragment showed better tenacity values than lines with the 'Gazul' fragment. These two fragments were successfully cloned and sequenced, representing different alleles of *B3-1* gene.

To further assess the potential of the molecular marker system in allele determination, we analyzed 29 bread wheat varieties, including standards for *Glu-B3* alleles (Liu et al., 2010; BMC Plant Biology). The amplification fragments were again scored (present/absent) in each variety. No correspondence between individual fragments and specific alleles was detected. However, it was possible to create a classification key where a series of choices between alternative fragments led to the identification of almost all alleles. The results obtained support the suitability of this molecular marker system for *Glu-B3* allele assignment and its likely transferability to *Glu-A3* and *Glu-D3* loci allele discrimination.

Grain quality of wheat wild relatives

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The wild relatives of wheat are an important source of genetic variation that allows the creation of new varieties with improved yield and quality. Protein content, sedimentation value and total phenolic content of 22 wild relatives from genetic collections of the Institute of Field and Vegetable Crops, Novi Sad, were analyzed during two years (2011-2012). The results were compared with several commercial cultivars (NS 40S-*Triticum aestivum*, Bambi-*Triticum compactum* and Nirvana-*Triticum spelta*). The protein content in the wild relatives ranged from 10.8 to 19.7%, while in the other genotypes was in the range of 9.8-15.5%. The highest protein content of all genotypes was recorded in Tr. dicoccum var. Ajar Spr. (19.0%), and the lowest in commercial variety Bambi (Tr. compactum) - 11%. Sedimentation value in the wild relatives ranged between 6.5-41.5 ml, and in the commercial genotypes between 11.7-48 ml. The highest average sedimentation value was found in commercial cultivar NS 40S (Tr. aestivum) - 44.5 ml, and the lowest in Tr. dicoccum var. Africanum (8.5 ml). Total phenol content in the wild relatives varied from 553.4 to 2839.7 μg catechin g^{-1}d.m, while in the other genotypes was in the range of 902.9-1398 μg catechin g^{-1}d.m. The highest average total phenol content was recorded in Tr. dicoccum var. Inerne D (2009.6 μg catechin g^{-1}d.m.), and the lowest in Tr. durum var. Melanopus (866.5 μg catechin g^{-1}d.m.). In the wild relatives significantly greater potential for expansion and improvement of the genetic basis of protein content was found, relative to sedimentation value. However, the low sedimentation value indicates lower protein quality, and therefore more attention should be paid to the contents of phenolics and their antioxidant activity. Further research should focus on other chemical indicators of a number of wild relatives, in order to perceive ability to use natural resources for the improvement of healthy and safe food.

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Winter cereals grain and straw suitability for heating in Latvia

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There is a global tendency to use alternative energy, thus partially replacing fossil energy with grains and straw which is a good alternative source of heating. The weather conditions in the Baltic States during the harvest period are variable and this variability affects the quality of grains. The grains that have a lower quality due to the weather conditions as well as straw can be used for the purposes of heating. The quality of raw materials for heating is defined by the ash content which is dependent on the chemical content of the raw materials.

The research was carried out in State Stende Cereal Breeding Institute from year 2009/2010 to year 2011/2012. During the research three rye ('Matador', 'Placido', 'Dankowskie Nowe') and triticale varieties ('SW Valentino', 'Dinaro', line 0002-26) were examined. The variables that were determined were grain and straw yield, crude ash content and the highest calorific value.

The straw and grain yield there were no substantial differences among the varieties and species. The grain and straw yield was affected by the year of cultivation and the genotype.

There were no substantial differences in crude ash content of straw among the species — it varied between 3.7 and 5.18%. The winter rye had the lowest crude ash content among all the winter cereals examined and the crude ash content was affected by the climate conditions.

The grains of triticale had the highest calorific value — 16359.07 kJ kg^{-1} on average. As regards the straw the highest calorific value was obtained from the straw of rye — 17410.94 kJ kg^{-1} on average during the three years of research.

When comparing the use of straw and grains for the heating, it can be noted that the grains were a more valuable raw material as it has a lower ash content and provides higher yield. The straw, however, provided higher calorific value. The straw had a higher ash content that encumbers the functioning of central heating boilers.

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Impact of environment conditions on variability of barley grain quality for food in Latvia

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Hulless barley research and development is now receiving more emphasis with potential for various end uses also in Latvia. It has been recognized as being more valuable and economic in food industry than covered barley because the interest of consumers in health strengthening and promoting food products is increasing. Hulless barley kernels contain high levels of β-glucans, it could be a good source of tocopherols and tocotrienols. Growing of hulles barley could open up new possibilities in use of barley species and increase the demand and interest of producers and growers including organic farming and organic products. The aim of the study was to evaluate the yield and grain quality of hulles barley (1000 kernel weight, crude protein, β-glucans, total phenolic, and α-tocopherol content) variation of three hulless spring barley genotypes under organic growing conditions. The field experiments were carried out at the State Stende Cereal Breeding Institute. Three hulless barley genotypes 'IC-360'; 'ST 1165'; ST 1185' and one hull barley variety 'Ansis' were studied during three years (2011 - 2013).

The variation of physical traits and macronutrients was mainly determined by genotype ($\eta 2$=4-51%) and year ($\eta 2$=35-76%) and to a lesser extent by interaction of factors.

Hulless barley included in this study showed medium high variability in β–glucan content (40.4 – 63.1 g kg^{-1}), α-tocopherol content (7.2 -9.0 mg kg^{-1}) and total phenolic content (145.1 – 183.6 mg GAE $100g^{-1}$ DW) between genotypes. Hulless line '1185' gave the highest grain yield, but 'IC – 360' formed significantly higher TKW, crude protein, α-tocopherol and β-glucan content. The best hulless breeding lines are selected for future usage in clinical investigations. Genotype 'IC 360' as new hulless barley variety 'Kornelija' since 2014 is registered in Latvia and Estonia.

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Qualitative and quantitative analysis of glutenin subunits in Korean common wheat cultivars by proteomic approaches

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Although it is known that the composition of HMW-GSs and LMW-GSs are important factor for end-product quality as bread, noodle and cookie, it is still not clear which HMW-GSs and LMW-GSs confer specific processing properties. In this study, to investigate distinctive glutenin proteins and expression level for characteristic processing properties, we carried out qualitative and quantitative analysis of gluetenin protein in noodle and bread wheat cultivars by two-dimensional electrophoresis. Unexpectedly, five LMW-GS spots were found to be expressed at a common position in all cultivars and these spots may play something in glutenin biosynthesis. Also we found LMS-GS spots to distinguish Korean wheat cultivars mostly used as noodle and western bread wheat cultivars. These spots may contribute to characteristic processing properties. The 2DE results for each cultivar will be used as reference map or protein marker discriminating wheat cultivars, wheat and rice, imported and Korean flour. For quantitative analysis of gluetenin, we calculated relative expression level of the HMW-GS, LMW-GS and HMW-GS/ LMW-GS ratio in each cultivar by 2DE. The results presented in this study provide new insight into relation of specific glutenin proteins and end-use quality and will be useful to choose elite breeding line for improvement of wheat flour quality.

Effects of genotypes and environments on yields and quality traits of popping maize (*Zea mays* L. *everta*) hybrids

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Kernels of popping maize, similar to other cereals, consists of three basic parts: pericarp, embryo and endosperm. The main trait by which popping maize differs from remaining maize types is an ability to form a large "flake" after the kernel ruptures as a response to heating. A popping volume represents the ratio between popped kernel and unpopped kernel and is expressed in cc/g. This trait is due to differences in shapes, sizes and compositions between popping maize and standard grain quality maize.

Effects of genotypes and environments on the yield and the popping volume were observed in 12 popping maize hybrids. Hybrids were sown in three-replicate trails according to the randomised complete-block design in three locations in 2012. The popping volume was established by a standard method (MWVT). The kernel size was determined on the basis of the number of kernels in 10 g. The popping volume is one of the most important parameters of quality, in addition to grain yield, which is economically the most important trait.

The analysis of variance showed the difference between hybrids and locations for observed traits and hybrid x location interactions. This suggests that not only genetic variance, but also, largely, ecological variance and variance of hybrid x location interaction affect the expression of observed traits. According to gained results, the correlation between the popping volume and the number of kernels in 10 g was positive. A weak negative correlation ($r = - 0.33^*$) was established between the number of kernels in 10 g and the grain yield, which means that genotypes with a greater number of kernels, i.e. smaller kernels express lower yields and vice versa. Furthermore, a very weak negative correlation ($r = - 0.02$) was determined between the popping volume and the grain yield. Breeding popping maize in the direction of yield increasing, often by increasing the weight of a single grain, including a higher proportion of soft endosperm, results in the popping volume decrease.

Suitability of maize hybrids for the industrial processing and silage production

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Maize is one of the most important crops, and as such, one of the most significant naturally renewable carbohydrate raw material and source of diverse products and energy. Moreover, regarding nutrition of domestic animals, maize is also one of the most important forage crops. In Serbia, maize is a traditionally mostly grown field crop. Although its role is not completely clear yet it can be freely stated that it is a crucial part of almost all aspects of our daily life, starting from a contemporary and healthy nutrition to a sustainable development and protection of the environment. Furthermore, an economic importance of maize is enormous.

According to the insight into the development of the research work on the improvement of maize utilisation, the objective of the present study was set up. The objective was to investigate and compare most widely grown maize hybrids in Serbia for the industrial processing and silage production.

All investigated maize hybrids had very different grain chemical composition and physical properties which could provide various possibilities of their utilisation. Starch yields of studied maize hybrids ranged from 66.55% in ZP 758 to 63.48% in ZP 600, while the highest (11.54%), i.e. lowest (6.00%) gluten yields were detected in ZP 648, i.e. ZP 600, respectively. The highest bioethanol yield (87.84% of theoretical) and volumetric productivity ($1.87 gl^{-1}h^{-1}$) were obtained with hybrid ZP 434 and the lowest with ZP 611k. This range of grain quality parameters provides diverse possibilities in hybrid selection for a certain purpose and their uses in the industrial processing. Furthermore, the results indicate to significant differences in dry matter digestibility of the whole plant among different hybrids in the most optimal harvest stage. The dry matter digestibility of investigated silage maize hybrids ranged from 49.89 to 65.09%.

Genetic variation and association mapping of grain quality traits in elite winter wheat genotypes

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Wheat is the dominant crop in the Central and West Asia and North Africa (CWANA) regionwith current annual production of 112 million tones in approximately 55 million hectares.End-use of bread wheat grain in the region is primarily leavened bread produced industrially, in small bakeries or at home. The International Center for Agricultural Research in the Dry Areas (ICARDA) has as main aim to develop new high yielding wide adapted varieties, resistant to biotic and abiotic stresses and with good end-use quality. To successfully accomplish this objective, ICARDA wheat breeding program combine molecular and classical breeding techniques. Association mapping approach has proved to be a valid method to improve our knowledge regarding the genetic control of several quantitative traits and can help increasing the effectiveness of the breeding strategies through marker-assisted selection. In the present study, we investigated the yield and quality performance of 120 elite winter facultative wheat genotypesand the association of approximately 2000 polymorphic diversity array technology (DArT) markers with grain quality attributes in order to identify closely associated markers for possible use in marker-assisted selection. The results obtained showed wide genotypic and phenotypic diversity among the ICARDA elite winter and facultative wheat genotypes.Several lines showedhigh yield potential and drought tolerance with different quality attribute combinations.ICARDA's elite lines tend to carry high molecular weight glutenin subunits1 and 2* encoded in Glu-A1, 7+8 and 17+18 in Glu-B1 and 5+10 in Glu-D1,subunits reported to have positive effect on dough quality. More than 30 markers linked to quality-related quantitative trait loci were identified, some of which are reported for the first time. The QTLs identified should still be validated althoughthe data presented can be of use for breeders and molecular biologists to enhance genetic diversity, yield potential, drought toleranceand end-use quality of their breeding programs and improve selection efficiency in their breeding strategies.

Identifying genes involved in (1,3;1,4)-β-glucan synthesis

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An important component of barley (*Hordeum vulgare*) cell walls is (1,3;1,4)-β-glucan, a polymer that has proven health benefits in humans and that influences processability in the brewing industry. Studies so far have been looking at β-glucan content in grain and in leaves as it was thought to be important for primary cell wall and probably storage function in the grain. Recent studies in rice show a moderate amount of β-glucan in the stem, highlighting that its function is not clear. Genes of the cellulose synthase-like (*Csl*) *F* gene family as well as *CslH1* the only member of the *CslH* gene family have been shown to be involved in (1,3;1,4)-β-glucan synthesis but many aspects of the biosynthesis are still unclear. Examination of the sequence assembly of the barley genome has revealed the presence of an additional three *HvCslF* genes (*HvCslF11, HvCslF12* and *HvCslF13*) which may be involved in (1,3;1,4)-β-glucan synthesis. Using the extensive data on barley, available from different projects at the James Hutton Institute and the University of Dundee can help in understanding which genes are involved in (1,3;1,4)-β-glucan synthesis. Co-expression and eQTL analyses could be used to identify transcription factors and other enzymes which are possibly involved in the pathway for (1,3;1,4)-β-glucan biosynthesis.

Impact of amylose/amylopectin ratio to wheat bread-making quality

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Waxy wheat flour have unique characteristics like high water absorption, damaged starch content etc. These characterisitics are based on its amylose-free starch. Anyway, bread made from pure waxy flour have poorer functional properties. Aim of this work was to determine optimal blends of waxy/normal starch. Reconstituted flours were created using gluten, waxy starch, normal starch and different rates of normal/waxy starch. Water absorption, dough rheology, experimental bread baking and bread shelf-life were measured.

On the basis of obtained results it is posible to pronounce that with increased amylopectine content:

1) water absorption is higher and faster
2) shelf-life is significantly improved
3) bread volume is improved, but without clear trend – necessary to conduct more experiments
4) bread shape is changed, also without clear trend
5) it is more problematic to make a bread
6) blends of 20 % waxy and 80 % normal wheat look to be optimal for bread-making quality improvement.

Genotype x season effects on alcohol yield in a population of hull-less barley

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Hull-less barley has been developed as a feed crop for monogastric animals and remains an important food crop in some mountainous regions. Removing the diluting effect of the husk creates opportunities to exploit endosperm components such as beta-glucan, to increase soluble fibre, or starch for alcohol production. Hull-less lines, developed for food use, but with high alcohol yield, could thus be a potential feedstock for fuel ethanol, particularly as recent developments have permitted the conversion of beta-glucan to additional fermentable sugars. Alcohol yield is largely determined by starch content and accessibility, so results from malted grain should correlate with those obtained using extraneous enzymes on un-malted barley. A population of lines, generated by mutation in the hull-less cultivar Penthouse, was previously shown to vary for a range of quality traits. Predicted spirit yields (PSY), i.e. the estimated volume of alcohol from a tonne of malted barley, were therefore calculated for 37 of the lines, over two highly contrasting seasons. Over all lines, there was no significant correlation between seasons for PSY, but there were highly significant effects of genotype and season and a significant genotype x season interaction. Soluble nitrogen ratios (SNR) were higher in 2011 and PSY values slightly lower, suggesting that some over-modification may have occurred, but, across genotypes, there was no significant correlation between PSY and SNR in either season. Comparison of grain data between years showed the grain length:width ratio (L:W) to be generally higher in 2011, when there was also a significant negative correlation between PSY and both L:W and Thousand Grain Weight (TGW). PSY was not, however, significantly correlated with either TGW or L:W in 2008, nor was it associated with grain beta-glucan content. These results suggested that thinner grain may have reduced starch content or promoted some over-modification, leading to an overall reduction in PSY in 2011, when multiple regression showed L:W and TGW, in combination, to account for just over 20% of the variation in PSY. These data emphasised the need for assessment, over a range of sites and seasons, to identify lines with consistently high alcohol yields.

Phytochemical characterization of cereals accumulating anthocyanins with potential health beneficial effects

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The increase in care of healthy diet and pharmaceutical uses of food has brought renewed attention to quality traits such as flavor and health properties of crops. Specific traits include enhanced levels of compounds with possible health effects such as flavonoids, carotenoids and sterols. The most common cereals used worldwide, such as rice, wheat and barley, also include genotypes rich in seed anthocyanins.

To study the potential of anthocyanin-rich cereal grains to contribute to a healthy diet, seed materials are needed for animal feeding studies which differ only in the contents of anthocyanins, but not in other metabolic traits. We used a wheat mapping population generated by crossing purple-grained hexaploid wheat “purple” and the yellow-grained cultivar “Novosibirskaya 67”. Equal numbers of yellow and purple offsprings were randomly pooled for bulk propagation. Separate bulks with anthycanin free grains or anthocyanin-containing grains were harvested in 2012 and 2013. To demonstrate the metabolic equivalence of the bulks except their anthocyanin contents, we performed targeted and non-targeted metabolic analysis. As an example we found only slight differences in amino acid contents between anthocyanin containing bulks and anthocyanin free bulks of both years. We then studied the impact of pigment accumulation on the germination rate. Using artificial aging treatments prior to the assays, germination rates of two bulks were also compared. By the result that the germination percentage of anthocyanin containing bulk was observed 27% less than anthocyanin free bulk, we tentatively conclude that anthocyanins might have a negative effect on seed germination rate.

In addition to the analysis of the wheat mapping population, we studied the pigment content and quantified the amount of anthocyanins of 21 barley accessions, thereby noticed that several of the lines contained pigments which could not be extracted. We hypothesize that pro-anthocyanidins represent the fraction of non-soluble pigments. Four representative genotypes were selected for exploring pigment accumulation during seed development. LC-MS will be applied to identify and quantify phenolic compounds. RT-PCR will be used to quantify the expression of enzymes in both anthocyanin and proanthocyanidin biosynthetic pathways.

Grain productivity and quality of pea-hulless cereal intercrops in Latvian conditions

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Fodder production for both conventional and organic farming systems is a challenge on the farm, especially for animals demanding high quality grain concentrates. As pig and poultry production is protein-demanding, cereals are often combined with grain legumes. Among grain legumes, peas are most common in crop rotations and quite productive in temperate climate conditions, including Latvian climate. In Latvia, field pea (*Pisum sativum* L.) is traditionally most widely used as the green silage. It is usually grown in mixture with cereals to restrict lodging. One of the strategies to produce high protein yield can be achieved through combination of high crude protein concentration in each component in intercrops. Latvian cereal breeders offer new hulless genotypes to make mixtures with legumes as raw materials for concentrate feeds. Their suitability has not been extensively studied yet. Research has been carried out on spring hulless cereal-pea mixtures for grain which has been cultivated on four experimental sites: conventional and organic field rotations on two locations (in Stende and Priekuli) during 2013. Estimations on biological and harvested yield of four intercrops have been done on spring hulless cereals (barley 'Irbe', oat 'S-156') and field peas ('Selga', 'Almara'). Fractionating seed samples were analysed for dry matter, crude protein, amino acids, fat, crude fiber and ash after the harvest. Results indicated that intercropping combinations affected significantly many characteristics such as lodging, plant height, biological, harvested yield and grain biochemical composition. Average harvested yield level varied significantly between experimental locations due to different weather conditions. In all experimental sites the highest dry matter and protein yield showed pea 'Almara' and hulless barley 'Irbe' intercrop (with *max* value of protein yield 0.768 t ha^{-1}DM). The highest sum of essential amino acids in all experimental sites formed pea 'Almari' (66.0 g kg^{-1}DM) and hulless oat 'S-156' (37.4 g kg^{-1}DM). Pea-hulless cereal intercrops grown under organic conditions showed significantly higher protein quality.

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Session VI

Mapping, Cloning and Beyond

In silico identification and characterization of wheat 4AL - *Triticum militinae* introgression

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Alien introgressions to wheat are known to be a rich source of genes for crop improvement and can facilitate adaptation to abiotic and biotic stress and to a wide range of environmental conditions. However, knowledge of underlying mechanisms remains limited. A cross between the common wheat cultivar Tähti and tetraploid *Triticum militinae* yielded several introgression lines. One of which, line 8.1, confers improved powdery mildew resistance in both seedling and adult plant stage. The locus with a major contribution to the phenotype was identified in the long arm of chromosome 4A. In addition to the recent introgression, wheat chromosome 4A carries a pericentromeric inversion and two translocations from chromosome arms 5AL and 7BS. This complex structure makes this chromosome an interesting model for the study of chromosomal rearrangements which accompanied wheat evolution and domestication. We flow-sorted wheat chromosome arms 4AS and 4AL from cv. Chinese Spring and the long arm of chromosome 4A with the *T. militinae* translocation (4AL-TM), and sequenced their DNA by Illumina. Virtual gene order map (Genome-Zipper) was constructed using a 4A-specific consensus map of 624 DArT markers. The map comprises 2,395 ordered genes (653 and 1742 genes for 4AS and 4AL, respectively). This virtual gene order and a battery of comparative analyses allowed precise characterization of ancestral translocations on 4AL. The 5AL and 7BS translocations were delimited to two distinct regions comprising 337 and 532 genes, respectively. The same approach was used to localize the *T. militinae* introgression region. The introgression is located at the very end of 4AL telomeric region. While in cv. Chinese Spring, the collinear region comprises 383 genes, only 127 homologous genes were identified in the introgressed region. This study demonstrates the power of our approach for identification and characterization of chromosomal rearrangements. This work has been supported by the grant LO1204 from the National Program of Sustainability I, Czech Science Foundation (14-07164S), Czech Ministry of Education, Youth and Sports (OP VK CZ.1.07/2.3.00/20.0165), Internal Grant Agency of UP (PrF-2013-003), and by the Estonian Ministry of Agriculture.

Mapping QTL, genetic differentials and the effect of *Rht-B1* under organic and conventionally managed systems in the Attila × CDC Go spring wheat mapping population

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A randomly derived recombinant inbred line (RIL) population (n=163) from a cross between CIMMYT spring wheat cultivar Attila and the Canadian spring wheat cultivar CDC Go was used to map quantitative trait loci (QTL) affecting various agronomic and quality traits and to investigate the feasibility of organic wheat breeding by determining genetic differentials along with the effect of *Rht-B1* in paired organic and conventional management systems. Heritability estimates differed between systems for five of the nine traits including grain yield, tillers, plant height, kernel weight and grain protein content. Direct selection in each management system resulted in 50% or less lines/RILs selected in common for eight of the nine (except for flowering time) studied traits. Overall, we mapped 45 QTL for various agronomic and quality traits across organic and conventional management systems for three years. Most of these QTL were specific to the management system; however, consistent QTL for grain yield, test weight, kernel weight and days to flowering were mapped in both systems on chromosomes 6A, 1B, 3A and 5B, respectively. The effect of *Rht-B1* was more pronounced in organic systems where lines carrying wild type allele were taller, produced more grain yield with higher grain protein content and suppressed weed biomass to a greater extent than mutant types. The result of the present study suggest that differences exist between two management systems for QTL affects along with selection of superior genotypes for a specific system. Indirect selection of superior genotypes from one system to another will not result in the advancement of best possible lines. Therefore, breeding of spring wheat cultivars specifically for organically managed lands should be conducted on organic systems.

The peri-centromeric heterochromatin of barley restricts gene diversity and evolution but not gene expression

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Low-recombining peri-centromeric heterochromatin (LR-PCH) has been regarded as antagonistic to expression, diversity and evolution of genes, yet for barley this genomic region contains roughly a quarter of the genes of the species. In this study we have investigated the effects of LR-PCH residency upon transcription, gene diversity, duplication and loss.

Unexpectedly, we find no significant difference in average transcript level or developmental RNA specificity between the genes in barley LR-PCH and those from rest of the genome. In contrast, all of the evolutionary parameters studied here show evidence of compromised gene evolution in the heterochromatin. First, genes within the LR-PCH of wild barley show reduced diversity and significantly enhanced π_a/π_s ratios compared to the euchromatin, indicating that background selection and selective sweeps have led to a build-up of poorly-selected, protein-altering polymorphism which is a genetic burden for the species . Second, gene duplicates (ohnologue pairs) derived from the cereal whole genome duplication event *ca.* 60MYa have been completely eliminated from the barley peri-centromeric heterochromatin. Third, local gene duplication in the barley LR-PCH is reduced by 29% relative to the euchromatin. Thus, the heterochromatin of barley is a permissive environment for gene expression but restricts gene evolution and it is a paradox that barley tolerates such major evolutionary restrictions in such a large fraction of its genome.

Lastly, the barley LR-PCH contains many loci that are important for breeders but they are trapped in extended haplotypes which are extremely difficult to break down by crossing to reassort gene alleles for crop improvement. Our study shows that the LR-PCH of wild barley has considerably more diversity, both genic and haplotype, than the cultivar barley gene pool, which is extremely impoverished for allelic diversity in this genomic compartment. The LR-PCH of wild germplasm should therefore be considered as a highly promising source of new gene diversity for crop improvement, not just for barley but for all the Triticeae cereals.

Genetic mapping of winter rye (*Secale cereale* L.) based on SNP markers

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Rye (*Secale cereale* L.) is an important crop in Middle and Eastern European countries. It is resistant to biotic and abiotic stresses and can be cultivated on poor soils. An interest in its breeding is mainly due to the evaluation of hybrids based on cytoplasmic male sterility phenomenon. Most of the modern hybrid varieties are based on pampa cytoplasm. It is difficult for restoration and thus, efficient restorer lines are needed. It is assumed that the most important genes responsible for pollen restoration are located on the chromosomes 1R and 4R. Although tightly linked markers of that gene were evaluated, they are hardly available for polish breeding companies.

The attempts were undertaken to construct dense genetic maps using two mapping populations (RIL). The maps were saturated with either DArTseq or GBS markers. More than 1500 markers were mapped in case of both populations. Composite interval mapping allowed identification of markers linked with pollen fertility restoration genes for future breeding programs.

Genetic mapping of *Triticale* with SNP markers

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Triticale, is a hybrid species that originated from a cross between wheat and rye. Breeding programs are focused on the development of new forms that have i.e. high yield potential, resistance against disease and winter hardiness. However, progress is limited due to lack of molecular markers that could speed up selection process. Such markers, if combined with the knowledge of the chromosomal location of the QTLs coding for traits of interests, would be highly valuable. The available well saturated genetic maps were evaluated based on DArT markers. With the development of next-generation sequencing technology new marker types, such as DArTseq or GBS become available. In comparison to DArTs, sequencing markers are based on SNPs that are mostly co-dominant. Moreover, such markers could be generated in large numbers what may allow highly dense map construction. Such maps may be a very useful tool for selection programs.

Genetic mapping using thousands of markers may be complicated using available commercial software. We have tested MultiPoint software that is dedicated for such analyses. Two biparental mapping populations (DB1xRB1 and BED-301-2013 114(5)-2-1 x BED-302-2013 BORWO) were profiled with SNPs and genetic maps were constructed. The genetic maps were verified with R/qtl under R-CRAN. The maps were used to map some agronomically important traits.

Mapping QTLs for grain protein content and grain yield components in durum wheat

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Grain protein content (GPC) in durum wheat (*Triticum turgidum* var. *durum*) is negatively correlated with grain yield. To evaluate possible genetic interrelationships between GPC and grain yield per spike, thousand-kernel weight and kernel number per spike, quantitative trait loci (QTL) for GPC were mapped using GPC adjusted data in a covariance analysis on yield components. Phenotypic data were evaluated in a segregating population of 120 recombinant inbred lines derived from crossing the elite cultivars Svevo and Ciccio. The material was tested at five environments in southern Italy. QTLs were determined by composite interval mapping based on the Svevo x Ciccio linkage map described in Gadaleta et al. (2009) and integrated with DArT markers. The close relationship between GPC and yield components was reflected in the negative correlation between the traits and in the reduction of variance when GPC values were adjusted to yield components. Ten independent genomic regions involved in the expression of GPC were detected, six of which were associated with QTLs for one or more grain yield components. QTL alleles with increased GPC effects were associated with QTL alleles with decreased effects on one or more yield component traits, or vice versa (i.e. the allelic effects were in opposite direction). Four QTLs for GPC showed always significant effects, and these QTLs should represent genes that influence GPC independently from variation in the yield components. Such genes are of special interest in wheat breeding since they would allow to increase GPC without a concomitant decrease in grain yield.

The identification of direct target genes of the wheat transcription factor *NAM-B1* using ChIP-seq

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NAM-B1 is a NAC transcription factor which is an important regulator of wheat grain nutrient content and monocarpic senescence. *NAM-B1* was originally cloned as the gene underlying the *Gpc-B1* QTL which increases grain protein content. It was also found that *NAM-B1* affects grain micronutrient content, particularly iron and zinc which are of great relevance to human health. These changes to grain nutrient content are related to a delay in flag leaf senescence, suggesting that *NAM-B1* controls remobilisation of nutrients from this leaf to the developing grain.

To understand how *NAM-B1* controls nutrient remobilisation and senescence we have identified direct targets regulated by *NAM-B1* using ChIP-seq (Chromatin Immuno-Precipitation combined with next-generation sequencing). We first created transgenic wheat lines expressing native levels of NAM-B1 tagged with a FLAG peptide to enable a specific pull-down of NAM-B1. DNA libraries prepared from the bound chromatin were sequenced on an Illumina Hi-seq using 100bp paired-end reads. We aligned these reads to a custom reference sequence we have developed, which consists of gene-rich contigs from the IWGSC (International Wheat Genome Sequencing Consortium), ordered with reference to a Chinese Spring x Paragon mapping population. Genomic regions where NAM-B1 binds were identified using the peak calling software MACS2 with reference to control lines not expressing the FLAG tagged NAM-B1.

Using ChIP-seq we have identified several hundred potential binding sites of NAM-B1 which are common between five biological replicates. 82% of these binding sites are within 1kb of the open reading frame of the nearest gene suggesting a regulatory role for these identified regions. Experiments to validate binding are being carried out and an update will be provided. To produce a high confidence list of *NAM-B1* target genes we are comparing the genes identified by ChIP-seq to genes which are differentially expressed in wheat *NAM-B1* RNAi lines (identified by RNA-seq). We will identify TILLING mutants in a subset of high confidence target genes with interesting putative functions to further dissect the pathways of nutrient remobilisation, senescence and grain nutrient content.

Mapping of QTLs of agronomically valuable traits in spring wheat (*Triticum aestivum* L.) in different ecogeographical regions of Russia

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During 2005-2013 years a set of 110 recombinant inbred lines of a spring wheat mapping population (ITMI) obtained by crossing variety 'Opata 85' with a synthetic hexaploid wheat 'W7984', which was produced by crossing *Triticum tauschii* (Coss.) Schmahlh. [syn. *Aegilops tauschii* Coss., *Aegilops squarrosa* auct. non L.] (DD) and tetraploid wheat (AABB) cultivar 'Altar 84' were evaluated in five different ecogeographical regions of Russia. Thirty nine economically important traits that manifest themselves at different stages of growth have been examined in each ecogeographical location. A total of 386 quantitative trait loci (QTLs) with LOD scores above 2.5 were identified. We have determined 197 QTLs with LOD scores exceeding 3.0. The QTLs for the traits studied, mapped onto all 21 chromosomes, and manifested themselves under contrasting environmental conditions with varying degrees of reliability. It has been shown that the manifestation of identified QTLs can depend or not depend on the environment, but the evaluated quantitative traits interact and correlate with each other. A change in the limiting factor causes a change of the spectrum of genetic loci that determine the variability of traits. In addition, it was established that the number of genes controlling the same trait can be linked into blocks or localized on different chromosomes or arms and their activation, as we assume, is controlled by a gene-coordinator. It is also known that the genetic determinants of flowering and fructification traits occurring at later stages of development of higher plants are often linked with the genes of the morphology-viability (M-V) system, which influence the growth and viability of the organism during the early stages of development. Therefore, chromosomal loci should be viewed not as mechanical linkages of genes, but as some organic normalization, as a group of functionally related genes, or as blocks of coadapted genes. The relationships between identified homologous and homoeologous QTLs with known major genes or QTLs responsible for the manifestation of the studied traits in wheat or other Triticeae genera are discussed. The identified QTLs may be of interest for further experiments on genetic control of the corresponding agriculturally valuable traits and for marker assisted selection in wheat breeding.

QTL mapping in spring soft wheat (*Triticum aestivum* L.) grown with different doses of nitrogen

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Intensification of nitrogen nutrition of plants (especially – cereals) is quite important task aimed at raising the productivity of agricultural crops. Improved plants with efficient absorption and assimilation of nitrogen fertilizers have better potential to fair yield of grain with a high-quality protein. Today, creation of such plants is mediated by identification of relevant genes involved in these processes and subsequent introduction of them into recipient forms, or using direct methods such as marker assisted selection (MAS). In our experiment in spring soft wheat (*Triticum aestivum* L.) of ITMI mapping population, which was obtained as result of crossing of the spring wheat cultivar 'Opata 85' with 'W7984' a synthetic hexaploid wheat, obtained after hybridization of *Triticum tauschii* (Coss.) Schmahlh. [syn. *Aegilops tauschii* Coss., *Aegilops squarrosa* auct. non L.] (DD) and tetraploid wheat cultivar 'Altar 84' (AABB) grown with different doses of N (without any, with half and overall doses of nitrogen fertilizer) we have identified and localized 108 QTL (43 QTL with LOD $\geq$ 3 and 65 QTL with 3 > LOD $\geq$ 2). Significant correlations between established loci were detected. The complex estimation of compared average quantities on nitrogen doses was made by variance analysis with calculation of parameters of determinant variations. The coefficients of correlation permit to determine a character adjoined correlation between determinants and gradient of doses of nitrogen nutrition. The obtained data suggests that the combining of specific QTL, revealed in soils with low content of mineral nitrogen and which became in different ecological conditions, can be used for isolation of variants with stable crop capacity. We have also revealed genomic regions involved in control of mineral nitrogen metabolism, including formation of traits related to vegetative growth and grain yield of spring wheat, which data can be used in breeding programs using identification and practical transfer of allelic variants of genetic determinants for physiological and economically important traits.

Association mapping in wheat: 90,000 opportunities to tag your trait

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Genome-wide association mapping is one of a number of the next-generation of genetic mapping resources to have recently emerged in crop species. Here we use a collection of ~500 varieties of bread wheat (*Triticum aestivum* L.) genotyped with an Illumina iSelect 90,000 SNP array to perform power calculations and genome-wide association (GWA) scans for multiple traits. Significant marker-trait associations are presented, validating previously identified QTL, as well as identifying novel QTL. The resources generated present a powerful resource for the genetic dissection of traits in wheat, with germplasm available on request.

Anchoring of physical map from wheat chromosome arm 3DS

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Bread wheat is one of the most important world's crops. An important part of global genomic research focuses on generating a reference wheat genome sequence that will provide a launching pad for the application genomics tools in wheat improvement. Our project aims to sequence 321 Mbp of wheat chromosome arm 3DS. We have purified this arm by flow cytometric sorting, constructed 3DS-specific BAC library and built physical map comprising 657 contigs. A critical step for further use of the physical map is the ordering of contigs along the arm to support its sequence assembly. We used a novel strategy that anchores any marker available for the chromosome arm to the physical map on the base of alignment of Illumina reads from 3-D pools of MTP to the sequence of the marker. SNPs markers were identified in these sequences and BAC contigs were anchored to genetic map using F7 mapping population Chinese Spring x Renan. Because of high complexity of wheat genome and similarity of its three subgenomes, 3DS-specific ISBP markers surrounding SNP markers were designed to ensure reliable scoring of mapping population by SNP detection methods. Genetically mapped ISBP and SNP markers were used to order contigs of the physical map of 3DS arm.

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Investigating genetic diversity in wild barley (*Hordeum vulgare* ssp. *spontaneum*) using exome capture

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Present day cultivated barley (*Hordeum vulgare* ssp. *vulgare*) is a product of intensive breeding efforts and selection aimed at combining traits such as resistance to biotic and abiotic stresses. A byproduct of this approach however has been a reduction in the diversity of its gene pool. Wild barley (*H. vulgare* ssp. *spontaneum*) is a rich source of new beneficial alleles that can potentially be introgressed into cultivated barley to increase its genetic diversity and to improve its adaptation to changes in climate. Wild barley populations found to be naturally occurring in its centre of origin - the Fertile Crescent - have been previously selected and examined for their genetic diversity using molecular markers. In this study, we have further selected a subset of 84 wild accessions sampled from Israel possessing highly diverse responses to environmental variables including elevation, mid-day temperature, and mean annual rainfall. We performed exome capture, a more efficient and economical technique compared to whole genome sequencing, to identify nucleotide polymorphisms (SNPs and InDels) in genic regions. Analysis of SNP effects (synonymous vs. non-synonymous) and their association with environmental variables will be highly informative in identifying exonic regions as potential candidates for improving the environmental adaptation of cultivated barley.

Using LTC software for wheat physical mapping: Increasing contig lengths and MTP quality

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Recently, a new analytical system for physical contig assembly was developed as a part of *TriticeaeGenome* initiative and implemented in LTC software as an alternative or complementary tool to FPC. LTC provides more effective detection of problematic clones and clone overlaps and better ordering of clones within contigs. These features reduce the risks associated with chimerical contigs classically encountered with FPC. The effectiveness of LTC was checked on simulated data (based on sequenced genomes of maize and rice) and on real data of different species including wheat and barley. Systematic comparison of LTC and FPC physical maps demonstrates that LTC produces longer and more reliable contigs. In addition to *de novo* contig assembly and minimal tilling path (MTP) selection, LTC enables curing gaps, reviewing, reordering, and elongating of contigs and MTPs obtained by standard FPC package; it includes tools for simplification of positive clone identification (during the anchoring procedure) based on the list of positive pools. LTC can utilize available marker and sequence information in manual editing, verifying, and merging of contigs and MTPs. LTC also can be used for testing the quality of clone sequencing, checking overlaps of MTP clones at the sequence level and suggesting solutions for repairing the observed gaps.

Identification of novel markers for the dominant dwarfing gene *Ddw1* in rye by next-generation sequencing approaches

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Winter rye (*Secale cereale* L.) is a traditional cereal in Germany with versatile uses for human and animal nutrition as well as a substrate for bioenergy production. Rye breeding aims to develop new high yielding varieties which are, in view of the global climate change, more robust and less demanding in water and fertilizer. These efforts are directed to enhance the sustainability of rye production and to keep rye competitive in modern agricultural production systems. Lodging resistance ranks among the major breeding goals in rye. The main genetic approach to overcome lodging is a reduction of plant height by exploiting dwarfing mutants. In rye, the gibberellin sensitive dwarfing gene *Ddw1* has been used particularly in Eastern European breeding programs to improve lodging in population varieties. The implementation of *Ddw1* in the development of homozygous dwarf inbred lines for breeding of highly productive hybrid rye varieties is hampered, as an efficient and reliable method to distinguish homo- and heterozygous dwarf genotypes is not yet available. To overcome this limitation we have identified the *Ddw1*-orthologous regions in rice, barley as well as *Brachypodium* and established novel conserved ortholog set (COS) markers closely linked to *Ddw1*. The novel co-dominant COS markers enable to distinguish between homozygous and heterozygous dwarf genotypes and allow to predict *Ddw1* genotypes with a precision not feasible before. Transcription profiles for bulks of dwarf and tall genotypes using “Massive Analysis of cDNA Ends” (MACE), a reduced complexity RNA-seq approach, allowed us to identify and map single nucleotide polymorphisms (SNPs) in candidate gene sequences. We have integrated additional markers in the recently published transcript map of rye chromosome 5R based on a genotyping-by-sequencing approach and DArTseq as a complexity reduction method. The established sequence and molecular marker resources enable the implementation of *Ddw1* in practical hybrid rye breeding programs and provide molecular tools to break up the complex between *Ddw1* and undesired genes, the latter of which having a negative impact on agronomically important traits.

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The map-based sequence of the barley chromosomes 1H, 3H and 4H

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Barley is a major target for genome sequencing and analysis, especially in the context of establishing genomics-based crop improvement (IBSC, http://barleygenome.org). Although the barley gene-space was described recently, advancing to the complete genome sequence remains still challenging: The fraction of repetitive DNA is ~80%, and each of the seven cumbersome chromosomes equals on average about six complete *A. thaliana* genomes. Hence, for sequencing the individual barley chromosomes a clone-by-clone strategy was applied, which was based on a pre-existing Minimum Tiling Path (MTP) of overlapping BAC clones established for each chromosome (~8000 – 9500 BACs/chromosome). So far, shotgun sequencing of chromosomes 1H, 2H, 3H, 4H and 5H was completed for the most part in 2013 (IBSC). Work on the remaining chromosomes (6H, 7H) is still in progress and will be finished in 2014. Here, the sequencing approach applied to chromosomes 1H, 3H and 4H will be presented. Using shotgun sequencing in combination with custom-tailored multiplexing (up to 670 samples) about 30000 BACs were sequenced (Illumina HiSeq2000, MiSeq and Roche 454 XL+) and assembled. Furthermore, in order to scaffold sequence contigs, 8 kb mate-pair libraries (Nextera) from pooled MTP-BAC clones were employed. In the near future the assembled barley genome will represent the most advanced reference for genetics and breeding.

Microarray and hormone analysis of the calli derived from different organs and genotypes in barley

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Currently, only two genotypes, cv. Golden Promise and cv. Igri, are available in a stable transformation of barley (Harwood 2012). However, it is desirable that a wide range of genotypes can be applied to the transformation for the breeding and the functional analysis of genes. Our research group is working to elucidate the genetic factors which determine the success or failure of the transformation in barley, for the purpose of establishment of a simple method with the genotype-independent and also with high efficiency. As part of this project, we performed the gene expression analysis during the callus development and shoot regeneration as well as the endogenous hormone analysis of callus derived from the different organs and cultivars.

The expressions of the genes in calli derived from immature embryo of barley cv. Haruna Nijo, as a representative of inefficient cultivars for the transformation, and Golden Promise were comprehensively analyzed with Barley Gene Expression Microarray (Agilent Technologies). As a result, we identified over 4,000 genes which showed expression differences more than twice between these two cultivars during the callus development or shoot regeneration.

Then the levels of endogenous hormone in the calli derived from cotyledons, coleoptiles and roots of the juvenile plants, as well as immature embryo, from Golden Promise, Haruna Nijo and cv. Morex, inefficient for the transformation, were analyzed with LC/MS/MS. As a result, the callus derived from immature embryo of Golden Promise, which showed the highest ratio of the regeneration of green shoots, had the highest content of auxin, indoleacetic acid. On the other hand, obviously large contents of salicylic acid and abscisic acid were observed in the calli derived from cotyledons, coleoptiles and roots of Morex and the immature embryo of Haruna Nijo, respectively. It is expected that these phytohormones associate with a regeneration from callus negatively.

Grain size QTL region *QTgw.ipk-7D* in wheat: Sequence analysis and synteny to related grass species

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The previously described QTL for thousand-grain weight *QTgw.ipk-7D* associated with microsatellite marker *Xgwm1002-7D* was originally detected in a BC_2F_3 advanced backcross population of the winter wheat variety 'Prinz' and the synthetic wheat line W-7984 (lab designation: M6) (Huang et al., 2003). Nearly-isogenic lines (NILs) were developed carrying introgressions of M6 in the genetic background of 'Prinz' with varying sizes on chromosome 7DS. The BC_4F_3 NILs had a 10% increase in thousand-grain weight compared to the control group and the recurrent parent 'Prinz'. The same QTL was detected in another population of winter wheat 'Flair' and synthetic wheat 'XX86' (Huang et al. 2004). By using homozygous recombinant lines developed from both populations, it was possible to fine-map *QTgw.ipk-7D* to an interval of approx. 1 cM flanked by markers *barc126*, *wmc405* and *gwm44* on wheat chromosome arm 7DS. Based on a chromosome arm 7DS-specific BAC library, BACs covering the region of *QTgw.ipk-7D* were isolated and their sequences were obtained by 454 sequencing. New microsatellite markers were developed based on the resulting BAC sequences and have been used for anchoring the BACs to the genetic map. Finally the region of *QTgw.ipk-7D* was delimited to a physical genomic region carrying at least 12 predicted genes. A well conserved synteny to the genomic sequences of rice, *Brachypodium* and *Sorghum* was observed. A single BAC contig covering the respective genomic region in barley was identified and completely sequenced. A more detailed comparison of the barley sequence to wheat with respect to genome evolution is currently conducted.

To verify possible candidate genes, sequencing of the appropriate genes from parental lines and various NILs aiming to identify SNPs is ongoing.

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

Cloning of the homeotic gene *Laxatum.a* (*lax.a*) - prospects from an improving barley genomics infrastructure

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The barley mutant *laxatum.a* (*lax.a*) displays a set of pleiotropic phenotypic characteristics compared to the wild type: (i) increased spike length due to extended rachis internodes, (ii) awns with a very wide base, (iii) thin and exposed grains, (iv) as well as a homeotic conversion of the lodicules into two additional anthers. High resolution mapping in ~2000 F2 plants delimited the gene locus to a 0,2 cM target interval in a region which is characterized by reduced recombination. Thus, a large physical distance that comprises up to 200 genes required further mapping effort. On the basis of advanced genomic resources (IBSC, 2012) our aim was to test an adapted version of cloning-by-sequencing method in barley. By combining whole-genome-shotgun sequencing (WGS) data of the parental genotypes, RNA-seq data of phenotypic pools as well as exome capture (Mascher et al. 2013) re-sequencing of genetically closely linked *lax.a* recombinant plants a single candidate gene could be identified for *lax.a*. These results proof the feasibility of adapting high-throughput sequencing methodology for gene isolation in barley.

References

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Sequence ready physical map of bread wheat chromosome 4A

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Despite progress in next generation sequencing technologies sequencing of large and complex plant genomes like that of bread wheat is still a challenge. Size, complexity, and repetitive element content of the allohexaploid bread wheat (*Triticum aestivum* L., 17 Gb) genome are the main obstructions from acquiring a reference genome sequence. Dividing the wheat genomes to chromosomes or chromosomal arms is powerful means to overcome the difficulties. As part of the coordinated effort by the International Wheat Genome Sequencing Consortium, we have constructed physical map of chromosome 4A using BAC libraries from flow-sorted short (4AS) and long (4AL) chromosome arms. The fingerprinted BAC clones were assembled to physical maps using LTC (Linear Topology Contig) software and additional merging and elongating was facilitated by super-scaffolding tool of the LTC software. The 4AS library was assembled into 250 super-contigs and Minimum Tiling Path (MTP) consist of 4 422 clones. The 4AL physical map was assembled into 924 super-contigs and MTP contains of 8 369 clones. The physical maps represent 86% and 89% of 4AS and 4AL, respectively. 67 and 74 super-contigs with more than 100 clones were assembled for 4AS and 4AL respectively, with the longest super- contig comprising 998 clones. Three dimensional pools of the MTP were sequenced and used for in silico anchoring of the contigs. A total of 1780 DArT markers were used to construct GenomeZipper from the 4AS and 4AL survey sequences. Additionally, 54125 and 62656 low-copy markers were identified from the survey sequences of the 4AS and 4AL chromosome arms, respectively. All these resources facilitated anchoring 100% of 4AS contigs and 99% of 4AL contigs. In order to facilitate ordering the physical map contigs in pericentromeric region and regions with low recombination rate, a Radiation Hybrid panel was constructed for chromosome 4A and genotyped with 90k SNP Infinium chip. This work has been supported by the grant LO1204 from the National Program of Sustainability I, Internal Grant Agency PrF-2013-003, by the Czech Science Foundation (14-07164S) and by Estonian Ministry of Agriculture.

Map-based cloning the gene *albostrians* in barley

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The *albostrians* mutant of barley (*Hordeum vulgare* L.) originated from the two-rowed spring barley variety Haisa by means of x-ray irradiation. Seedlings of the mutant line 4205(M4205) can be either fully green, white (albino) or show green and white sectors over the entire plant. Progeny of a green homozygous mutant plant obtained by selfing will segregate into 10% green, 80% green-white striped and 10% white seedlings. Thus the wildtype gene product seems to be essential for the chloroplast development, however, only at a critical step of embryo/plant development. As in white sectors as well as fully albino M4205 plants chloroplasts contain no ribosomes, one may speculate that the gene *albostrians* is functional in the process of plastid transition and/or biogenesis of the plastid ribosome. By using a F2 mapping population derived from a cross between cv. 'Morex' x 'M4205', the gene *albostrians* was allocated on chromosome 7H and from the physical map of barley a single BAC contig carrying the candidate gene and both closest flanking markers was identified. In order to test the function of the candidate gene we are following a parallel approach of transgenic complementation and induction of novel alleles by mutagenesis. While constructs for transgene analysis are under construction we screened our available TILLING (Targeting Induced Local Lesions IN Genomes) population derived from cultivar Barke. 10,279 M2 plants were screened for all three exons of the *albostrians* candidate gene. One M2 plant carrying a mutation leading to a premature stop codon was identified. Phenotypic analysis of its M3 family reveals that all of the homozygous mutants show albino phenotype providing first evidence that the identified candidate gene is involved in the process of chloroplast development. Further functional characterization of the candidate gene is underway.

Construction of high density genetic map and QTL mapping of agronomic traits in diploid wheat

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Genetic map provides a framework for quantitative trait locus (QTL) mapping, molecular marker assisted selection, map-based cloning of genes, and genome sequence assembly. A high density genetic map was constructed using a recombinant inbred line (RIL) population between diploid wheat *Triticum boeoticum* (Tb) × *T. monococcum* (Tm). QTL analysis of the population identified 15 loci for agronomic traits including heading date, spike length, spikelet number per spike, plant height and type, etc. To saturate the map, Restriction-Associated DNA (RAD) sequencing was explored in the population.

The genetic map includes 640 DArT, SSR, RFLP, functional gene, protein and morphological markers (MM), and the total length is 713 cM, with a marker density of 1.1 cM/interval. The parental preferences of markers were found frequently across the genome. The population was evaluated in 2011-2013 growing seasons in Beijing, and the QTL analysis was performed with winQTLCart v2.5. Fifteen QTLs conferring to heading date, plant type, etc, were detected on five chromosomes, of which eight QTLs were located in chromosome 3A. To increase the marker density of the QTL flanking region on chromosome 3A, the *T. urartu* (A^uA^u) genome sequences were attempted to develop molecular markers and 10 SSR markers were incorporated into an 8.8- cM region covering the QTL for heading date in chromosome 3A.

To saturate map, genomic DNA of each lines were digested by *SbfI*, each of which was ligated to barcode adapter to discriminate different samples, then DNA from 16 samples were pooled into one library and sequenced at Illumina Hiseq2000 platform. SNP calling was performed using the RAD sequencing data of the RIL population and SNP map construction are carrying out.

In summary, a high-density genetic map of diploid wheat was constructed based on the genomic sequences, which is convenient for QTL analysis of complex traits. RAD technology facilitated the rapid discovery of thousands of SNPs and reduced the cost and time of marker development.

Towards an SNP-based consensus map of durum wheat

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High-density Single-Nucleotide Polymorphism (SNP) arrays are becoming the golden standard for genotyping, even in polyploid species with a large and complex genome such as durum wheat (*T. turgidum* spp. *durum*). While a growing collection of SNPs is available in bread wheat, limited information is available for durum wheat. Therefore, durum wheat genetics and breeding would greatly benefit from the availability of an SNP-based consensus map with robust links with bread wheat. The wheat Illumina iSelect 90k array (Akhunov et al. submitted) provides ca. 76,000 functional SNPs, 8,000 of which are durum-specific. This SNP array was used for genotyping six *T. durum* × *T. durum* mapping populations and four interspecific populations (*T. durum* × *T. dicoccum* or *T. dicoccoides*) whose genetic maps were already constructed with microsatellite and/or DArT® markers. The intraspecific populations allowed us to map from 5,500 up to 6,200 SNPs/population with an average SNP density of ca. 1 SNP/0.3 cM. These populations differed in local marker density, suggesting the presence of extended identical-by-descent regions, major selective sweeps and/or variation in crossing-over frequency. The *T. durum* × *T. dicoccum* or *T. dicoccoides* populations showed higher polymorphism, with up to 12,000 SNPs/population. With a total of 13 mapping populations used for producing a consensus map (three additional populations genotyped with SSR, DArT or the wheat Illumina iSelect 9k array were also considered) it has been possible to produce a a preliminary framework consensus map of anchor markers (present in two or more populations) of 16,688 markers for a total map length of 2,710 cM. The map also included additional 13,957 unique markers, bringing the final number of mapped markers to 30,645. While the order of SNP loci was highly consistent between the single maps as well as between the component and the consensus maps, a small number of loci deviated from perfect collinearity. The progress on the assembly of the consensus map for other chromosomes will be reported.

Mapping of loci determining agronomically important traits in spring soft wheat (*Triticum aestivum* L.) under controlled conditions

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In this study a set of 110 recombinant inbred lines of the International Triticeae Mapping Initiative (ITMI) mapping population including the parental forms 'Opata 85' and the synthetic hexaploid wheat 'W7984' was evaluated under controlled conditions. Two experiments were conducted. Wheat plants were grown under 16th photoperiod and different levels of illumination (40±5 W/m^2 FAR and 50±5 W/m^2 FAR in first and second experiment, respectively) and temperatures (20±5ºC - day/night and 24±5ºC - day/night, in first and second experiment, respectively) during the whole vegetation period. All other conditions were identical in both experiments where 19 agronomical important traits were evaluated. A total of 63 QTL with LOD-score above 2.5 were identified and mapped in both experiments. For 9 identified QTL the LOD-score was ≥ 4.0. Stability of QTL for investigated traits was checked in both experiments. Significant correlations between established loci were detected. For a number a traits the QTL position did not remain the same from one experiment to another. Obtained results may be used for directed marker assisted selection.

Genetic analysis of wheat domestication

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Wheat provides about one fifth of the calories consumed by the human population and is considered to be one of the most important crops. Wheat is occupying the widest growing areas globally, however, wild emmer [*T. turgidum* ssp. *dicoccoides* (Körn.) Thell.] which is the progenitor of modern wheat varieties is growing exclusively in the Fertile Crescent. The accepted view is that the domestication of many important staple crops, including tetraploid wheat, originated in this area 10,000 years ago with the transition from hunter- gatherer society to an agrarian one. This domestication process involved significant genetic changes in genes controlling several spike- related traits leading to the emergence of plants with non-brittle rachis, soft glumes and free-threshing seeds. These favorable traits were selected during domestication and subsequently became fixed in the cultivated population. In order to identify genes controlling domestication-related traits, a high density genetic–map of tetraploid wheat was constructed using 7,475 markers and 140 F_6 recombinant inbred lines (RILs) derived from a cross between elite durum wheat cultivar 'Svevo' and wild emmer wheat accession 'Zavitan'. This population was also phenotyped in the field for domestication related traits. QTL analysis with the MultiQTL package revealed several loci controlling rachis brittleness (*Br*), glume tenacity and threshabilty (*Q*, *Tg*, *Sog*). For example, four *Br* loci were identified on chromosomes 2A, 2B, 3A, and 3B to confidence intervals of 8, 11, 2, and 10 cM, respectively. These loci were previously mapped in other studies, therefore confirming the integrity of our genetic map. To the best of our knowledge, this is the first report of the simultaneous mapping of these four important domestication related loci using one genetic population, hence confirming the complex genetic model involved in spike brittleness. This also suggests that our mapping population captures a major part of the genetic variation between wild and domesticated wheat, and hence can serve as an important tool to unravel the genetic mechanisms controlling wheat domestication.

Development of a new SSR-based linkage map of bread wheat including 59 new SSR loci

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Although many mapping populations and their genetic maps for bread wheat are available, the wheat breeders and geneticists are primarily interested in their own mapping population. This research aimed to develop a new SSR-based genetic linkage map of bread wheat. Segregating population including 143 F_2 individuals derived from an intraspecific cross between HTRI 11712 × HTRI 105, two bread wheat accessions from Pakistan and Sweden, respectively. The parental lines were analyzed with 666 simple sequence repeat (SSR) primer pairs and 398 SSR including 372 GWM, 5 GDM, and 21 BARC primer pairs, on average about 60 percent, revealed polymorphism. Twenty five out of the total tested primer pairs showed null alleles in the both parental lines. According to the positions and genetic distances of the polymorphic loci a total of 273 microsatellite primers pairs including 247 GWM, 21 BARC, and 5 GDM were selected to genotype all of the 143 F_2 plants. A total of 293 polymorphic loci were mapped associated with 19 chromosomes except chromosomes 6D and 4D, with a total map length of 2,711 cM. There was on average one locus per each 9.2 cM on this map. Markers in the current genetic map verified the two well-known reciprocal nonhomoeologous translocations of 4AL/5AL, and 4AL/7BS in wheat. Most of the markers maintained their position and order along the linkage groups as presented in the reference ITMI linkage map. Importantly, in total 76 loci (including 27 extra loci and 45 first time mapped loci) out of 293 mapped loci were considered as new loci compared to the ITMI map. Since 13 of these loci were reported earlier by other genetic maps, therefore the current linkage map contains 59 newly reported loci which increased the coverage and density of existing wheat microsatellite genetic linkage maps. Above all, because the population size in the current study is doubled compared to the ITMI map, and there are only less than one percent missing values in the current genotypic data set, therefore, the current map has more accuracy regarding to the SSR loci that are common between them.

Mapping two new QTLs for days to flowering in bread wheat

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The present study was conducted to map QTL for days to flowering in bread wheat. Two cultivated accessions were selected as parental lines; one from Pakistan and the other from Sweden. Mapping population was developed from a single F_1 plant. Phenotyping was carried out on the 133 $F_{2:3}$ families through two field experiments at IPK-Gatersleben in 2004 and 2005. The genetic map including 248 loci were constructed and applied for the QTL analysis. Composite Interval Mapping revealed eight QTLs for days to flowering on chromosomes 1B, 2D, 3A, 5A (two QTLs on 5AL), 5D, 7D, and 7B. The R^2 for a single QTL ranged from 7.7 to 14.7%. Interestingly, two QTLs (*QDtf.ipk-5A.1* and *QDtf.ipk-5A.2*) were identified on the chromosome 5AL in 2004. Snape et al.(2001) suggested the existence of loci for vernalization on the chromosomes 4BL, 4DL, and 5AL (because of the 4AL/5AL translocation) due to the synteny with locus *Vrn-H2* on chromosome 4H of barley and also the work of (Dubcovsky et al., 1998) who mapped two loci *Vrn-A*^{m}*1* and *Vrn-A*^{m}*2* on the proximal and distal regions of chromosome 5A^m of *T. monococcum*, respectively suggesting the existence of polymorphisms for *Vrn-2* series loci in wheat, the orthologous of *Vrn-H2* from barley. Therefore, the QTL (*QDtf.ipk-5A.2*) which co-localized with QTL for awnedness on distal part of the chromosome 5AL, on the *B1* locus, is most likely the one that was reported in diploid wheat but was not mapped in bread wheat. Khlestkina et al. (2009) showed while photoperiodic response gene located near to the end of chromosome 7BS, the vernalization gene was located more close to the centromere similar to the mapped QTL on homoeologous chromosome 7DS in the present study, the region far from 7BS/4AL translocation segment. Therefore, the QTL on 7DS (*QDtf.ipk-7D*) probably is for vernalization response. The QTL *QDtf.ipk-5A.2* showed the highest value for LOD score (7.38) whereas *QDtf.ipk-7D* showed the highest amount for R^2 (24.7%). Finaly, two QTLs on the chromosome 5AL (*QDtf.ipk-5A.2*) and chromosome 7DS (*QDtf.ipk-7D*) both most likely for vernalization response (*Vrn*) were reported for the first time here.

Restriction polymorphism of amaranth starch synthesis genes in relation to radiation mutagenesis

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The aim of the study was to analyze the restriction polymorphism of *GBSS*I (Granule-bound Starch Synthase) and *SSS*I (Soluble Starch Synthase) genes in amaranth mutant lines and control samples. The presence of *GBSS*I (accession number AB456685), responsible for amylase synthesis and *SSS*I (accession number AB626804), amylopectin synthesis responsible gene, was detected in all of the mutant and control samples. *GBSS*I gene was amplified in three and *SSS*I gene in seven segments. Restriction endonucleases *Aci*I, *BsaJ*I, *Fat*I, *EcoR*I, *BamH*I, *Pst*I, *Hpa*II, *Pci*I were used as a tool for evaluating the polymorphisms of restriction sites. GBSSI gene was digested by *Hpa*II and *Pci*I restriction endonucleases. Two *Pci*I and three *Hpa*II restriction sites were evaluated, recording no changes in the restriction pattern. Six types of endonucleases were used to evaluate *SSS*I gene. The analysis of *Aci*I, *BamH*I, *EcoR*I, *Pst*I restriction sites did not show any polymorphisms. However, restriction profile changes were recorded when *BsaJ*I and *Fat*I were used in C 15/1 and C/236/1 mutant lines. Restriction polymorphism analysis represents an effective tool of nucleotide variability identification of plant genome at the inter-specific level.

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Genomics of alien gene transfer in wheat

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Wheat, maize and rice are the three most important crops worldwide. Wheat itself is feeding nearly half of the world's population (40%) and provides 20% of the total food calories and proteins in human nutrition. There is a big concern that productivity and quality of cultivated wheat continue to increase in order to satisfy the need for food by the growing human population. One of the strategies for wheat improvement is to enrich the existing gene pool of cultivated wheat by introducing favourable alleles, genes and gene complexes from wild and close relatives. Although alien gene introgression represents a powerful approach for crop improvement, the knowledge of underlying biological mechanisms remains limited. This project aims to provide novel information on the interaction between a host genome and introgressed chromosomes and their parts, both at genome structure and transcriptome levels. We have chosen wheat-barley ditelosomic addition line 7HL as a model system. The line was obtained after interspecific hybridization between wheat cv. Chinese Spring and barley cv. Betzes. The addition line 7HL contains the full set of wheat chromosomes and a pair of long arms of barley chromosome 7H, and exhibits interesting agronomic traits such as salt tolerance, earliness and high β-glucan content. We have sequenced transcriptomes of both parental species and the addition line 7HL to compare gene expression between the ditelosomic line and the parents. One of the difficulties is to discriminate homoeologous transcripts from duplicated copies in the wheat genome, and to distinguish barley transcripts from their host relatives in the ditelosomic line background. The results of this study should provide new information to understand the regulation of the alien genes in the host genome, and reciprocally the impact of the additional alien genes copies on the expression of their host homoeologues.

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Six-rowed spike genes regulatory network in barley

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Barley (*Hordeum vulgare*) spikes are developmentally switched from two-rowed to six-rowed by a single recessive gene, *Six-rowed spike 1* (*vrs1*), which encodes a homeodomain-leucine zipper I class transcription factor. Loss of *Vrs1* function converts the sterile lateral spikelets seen in the two-rowed barley spike into fully fertile spikelets. *Vrs1* is expressed exclusively in the pistil, lemma, palea and lodicule of lateral spikelets. VRS1 protein, which is localized in nuclear, is also expressed in lateral spikelets and acts as a transcriptional activator. Expression analysis of six-rowed spike mutants that are nonallelic to *vrs1* showed that *Vrs1* expression was up-regulated by *Vrs4*, which is an orthologue of maize transcription factor *RAMOSA2*. However, little is known about the downstream genes regulated by *Vrs1*. Here, we conducted chromatin immunoprecipitation combined with high throughput sequencing (ChIP-seq) to identify direct target DNA sequences of VRS1 transcription factor. Moreover, genome wide expression analysis (RNA-seq) in immature spikes of two-rowed spike (wild type) and six-rowed spike (mutant) was carried out. These results provide available information to reveal veritable target of *Vrs1* and contribute to understanding of the regulatory network for the development of the lateral florets.

Analysis of the genomic structure of a wheat NIL population segregating for resistance to glume blotch with a 90k ILLUMINA SNP chip

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Single nucleotide polymorphism (SNP) markers have recently become highly relevant for genetic analysis in wheat because of new SNP genotyping technologies like the Illumina Golden-Gate Assay. There, SNP markers allow high-throughput and cost-effective genotyping, even in the polyploid wheat genome.

We used the wheat Illumina Golden-Gate Infinium array 90K [1] to unravel the genetic structure of a population of 89 near-isogenic lines (28 BC3F8 and 61 BC3F7) derived from the introgression of a specific genomic region coming from Arina (resistant) into Forno (susceptible) involved in quantitative resistance to Stagonospora nodorum glume blotch (SNG), a necrotophic fungal disease affecting bread wheat spikes [2]. We wanted to know the extent and size of genomic fragments derived from the donor line in the NILs.

From a dataset of 81.587 SNPs, 14.158 SNP markers (17.35%) failed in all samples, leaving a total of 67.418 (82.63%) functional SNPs. The reproducibility of the SNP chip was confirmed given the tiny % of non-conclusive SNPs (13, 0.016%) comparing replicates coming from the same DNA sample for both parents.

Among the functional SNPs, 8.407 (12.46%) were polymorphic between Arina and Forno. This is surprisingly high if compared with the percentage of polymorphism observed between winter wheat Arina and the spring wheat cultivar Chinese Spring (11.000 polymorphic SNPs), two wheat varieties with an obviously very different origin. Thus, the SNP chip reveals a large number of genetic differences, even between elite winter wheat varieties derived from the same breeding program.

Most of the SNPs polymorphic between Arina and Forno (8.363, 12.40%) were polymorphic also across the NIL population. Examining the allelic composition of the polymorphic SNPs within the population, 66.63% of SNPs shared less than 6.25% and approximately 27% of SNPs share > 25% of allelic composition with Arina. We will also present data on over- and underrepresentation of genomic regions in the NILs which might be indicative of some selection during population development. Furthermore, we will present the analysis of genetic size and frequency of specific introgressions in the NILs to determine the molecular nature of such a population in wheat.

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Fine-tuning the Green Revolution: Characterization of second site suppressor mutations in the *DELLA Rht-B1c* gene of wheat (*Triticum aestivum*)

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Since the Green Revolution in the 1960s, wheat productivity has been boosted by the introduction of the height-reducing Rht-B1b and Rht-D1b alleles, which encode mutant forms of the growth-constraining DELLA proteins. These mutant forms are less sensitive to the DELLA degradation mechanism initiated by binding of gibberellin (GA) to its receptor. Although it is known how GA controls the level of DELLAs within the cell, it is currently unclear how DELLAs constrain growth. Furthermore, the widely used Rht-B1b and Rht-D1b alleles are associated with some undesirable agronomic features that limit their value, particularly in challenging environments. Therefore, this research focuses on (i) characterizing novel semi-dwarfing alleles in wheat, and (ii) elucidating interactors of the DELLA protein that translate DELLA levels into wheat growth responses.

Novel second-site mutations were generated within the strongly dwarfing Rht-B1c allele. The novel mutants were characterized by a partial release of growth constraint, i.e. the mutants were all taller than the parental dwarf containing the Rht-B1c allele, with some as tall as the GA sensitive wild-type (Chandler & Harding 2013). In-depth phenotyping has revealed a similar trend for other GA influenced traits, such as coleoptile length and seed dormancy. Grouping of the mutants based on these phenotypic data showed that the intensity of the release of the DELLA-imposed constraint on GA responses depended on the type and position of the second-site mutation. This interesting observation forms the basis to further investigate the effect of the mutations on protein-protein interactions and nuclear localization.

Role of *Vrs1* and *Hox2* in barley spikelet development new findings and insights

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The spike architecture (row phenotype) of barley is controlled by the VRS1, a HD-ZIP I family transcription factor. *Vrs1* (*HvHox1*) is a paralog of *HvHox2* which is conserved among cereals, whereas *Vrs1* might have acquired its current function during the evolution of barley. *Vrs1* expression was found only in the lateral spikelets of two-rowed barley and might negatively regulate its development, whereas in six-rowed barley either the gene is absent or mutated, resulting in a non-functional protein. Contrastingly, *HvHox2* expression was found in leaves, roots and vascular bundles of developing spikes. Though the expression locations of both genes were discovered, the functional significance of these genes in spikelet development is still unclear.

We fused the *Vrs1* and *Hox2* promoter to eGFP and studied their expression pattern during spikelet development of two-rowed transgenic barley. Surprisingly, we found *Vrs1* promoter activity in both central and lateral spikelet meristems. The results were confirmed by quantifying the *Vrs1* transcripts in qRT-PCR from both tissues of *Hordeum vulgare cv. Bonus* (two-rowed barley). Also, we observed *Vrs1* promoter activity in stomata and vascular bundles of transgenic barley leaves. Similarly, we observed *Hox2* promoter activity in both central and lateral spikelet meristems and matured reproductive organs. This result has also been confirmed by qRT-PCR. Additionally, we swapped both the promoters to identify the regulatory targets and their significance in spikelet fertility. These findings pave way for the new insights into the role of *Vrs1* and *Hox2* in barley spikelet development.

An *Avena* A-genome zipper and its application to hexaploid oat breeding

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An Avena A-genome zipper has been constructed based on sequencing of parents and inbred progeny from an interspecific cross between wild and domesticated diploid species (*Avena atlantica* x *A. strigosa*, AxS). 1.55Gb of *A. atlantica* contigs with an N50 of 11.8kb have been mapped to intervals averaging 1% of the total AxS map length. Some 30,552 genes have been predicted from the total assembly with high confidence and annotated, with CEGMA evaluation indicating that 96% of expected genes are present with 85% being intact and contained within a single contig. Almost 95% of the assembled gene content appears to have been mapped. An Avena C-genome assembly is being constructed from the diploid *A. ventricosa*. The A- and C-genome references are being used to anchor SNP and genotype-by-sequencing (GBS) tags developed by the North American Collaborative Oat Research Enterprise (CORE), to improve identification of candidate genes and application across populations.

Genetic mapping of the *labile* (*lab*) gene: A recessive locus causing irregular spikelet fertility in *labile*-barley (*Hordeum vulgare* convar. *labile*)

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The *labile*-barley displays a variable number of fertile spikelets at each rachis internode (0-3 fertile spikelets/rachis internode) which is intermediate between that observed in two- or six-rowed types. Previous re-sequencing of *Vrs1* in 219 *labile*-barley (*Hordeum vulgare* L. convar. *labile*) accessions showed that all carried a six-rowed specific allele. We therefore hypothesized that this seemingly random reduction in spikelet fertility is most likely caused by the *labile* (*lab*) locus, which we aimed to phenotypically and genetically define. Here we report a detailed phenotypic analysis of spikelet fertility in *labile*-barleys in comparison to two- and six-rowed genotypes using SEM analysis. We found that the first visible morphological deviation occurred during the stamen primordium stage, when we regularly observed the appearance of arrested central floral primordia in *labile*- but not in two- or six-rowed barleys. At late stamen and early awn primordium stages, lateral florets in two-rowed and only some in *labile*-barley showed retarded development and reduction in size compared with fully fertile lateral florets in six-rowed barley. We used two F_2 mapping populations to generate whole genome genetic linkage maps and ultimately locate the *lab* locus as a recessive Mendelian trait to a 4.5 - 5.8 cM interval at approximately 80cM on chromosome 5HL. Our results will help identifying the role of the *lab* gene in relation to other spikelet fertility factors in barley.

Genetic architecture of heading date in European winter wheat

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The genetic dissection of molecular mechanisms underlying yield, plant height and heading date are important for the development of high-yielding bread wheat (*Triticum aestivum* L.) varieties. Heading date (HD) is one of the critical traits for the adaptation of wheat to diverse climatic environments and cultivation in various regions and cropping seasons. The results of a genome-wide association study (GWAS) for heading date will be presented using a panel of 358 European winter wheat varieties plus 14 spring wheat varieties. The phenotypic data are based on the evaluation of HD in field tests in eight environments. Genotyping data consisted of 770 microsatellite loci and 7934 SNP markers derived from the 90K iSelect wheat chip covering the entire wheat genome. Best linear unbiased estimations (BLUEs) were calculated across all trials and ranged from 142.5 to 159.6 days after the 1st of January with an average value of 151.4. In total 358 SSR and 2983 SNP marker-trait associations (MTAs) were detected, including only associations with a $-\log_{10}$ (P-value) ≥ 3.0. A total of 90 SSR and 438 SNP marker-trait associations remained significant after Bonferroni correction for multiple testing. For the photoperiodism gene *Ppd*-D1, which was genotyped in all varieties, also highly significant MTAs were detected. Reliable associations were found on all chromosomes with the highest number of MTAs on chromosome 5B. Linear regression showed a clear dependence of the HD score BLUEs on the number of favourable alleles (decreasing HD) and unfavourable alleles (increasing HD) per variety, i. e. genotypes with a higher number of favourable or less unfavourable alleles showed lower HD and therefore flowered earlier. For the vernalization gene *Vrn-A2* co-locating MTAs were detected on chromosome 5A as well as for the photoperiodism genes *Ppd-A1* and *Ppd-B1* on chromosomes 2A and 2B. After the construction of an integrated map for the SSR and SNP markers and by exploiting the synteny to already sequenced species such as rice and *Brachypodium distachyon* we were able to demonstrate that a marker locus on wheat chromosome 5BL showing homology to the rice photoperiodism gene *Hd6* played a significant role in determination of the heading date in wheat.

Session VII

Future Challenges and Innovations

Regulatory role of microRNA172 in barley flower opening

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In a typical grass flower, lodicules swell when flowering to open the floret, referring to chasmogamy or non-cleistogamy. Flowers which show cleistogamy remain closed during pollen shedding and ensure self-pollination. Cleistogamy provides a means of escape from cereal head blight infection and minimizes pollen-mediated gene flow, while non-cleistogamy is essential for F_1 hybrid production. Cleistogamy in barley is controlled by a single recessive gene, *cleistogamy1* (*cly1*). We have isolated *cly1* as being an APETALA2 transcription factor, an expected target of microRNA172, whose expression was concentrated within the lodicule primordia (Nair et al. 2010, PNAS). A single base change at miR172 target site in *cly1* allele results in smaller lodicules and cleistogamous phenotype. The loss of miR172 target site prevents miRNA-mediated cleavage in cleistogamous barley. We have identified wheat homeologs of *HvAP2*, which determine the flowering type in wheat (Ning et al. 2013, TAG). We hypothesized that HvAP2 protein suppresses lodicule development and miR172s regulate *HvAP2* to reduce the abundance of HvAP2 to confer non-cleistogamy in barley and wheat.

miR172 members are differentially regulated to perform different roles, conserved between monocots and eudicots, and may acquire some specialized species-specific functions. Our small RNA sequencing and in silico data analysis supported our hypothesis that miR172s are the main regulator of *HvAP2* at post-transcriptional level. We determined the expression profile of the executed miR172s by qPCR and in situ hybridization. Our in situ analyses showed that miR172 and *HvAP2* express in a highly overlapping domain. We identified the candidate *Hv-miR172* genes that could produce the executed miR172s. *Hv-miR172s* expression profile suggests the identity of *miR172* gene regulating *HvAP2* for barley flower opening. Further analysis to determine the miR172-mediated cleavage or translation repression of *HvAP2* in lodicules is ongoing. This study revealed a direct evidence of interaction between *HvAP2* and miR172s for lodicule development and flower opening. It is speculated that miR172s play a specialized grass-specific function in flower opening. This information would be implicated to the regulation of *TaAP2* in wheat, *SHAT1* in rice, and *AP2* orthologs in other cereals for normal flower opening.

Development of new oat varieties non-toxic for the coeliac population

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The treatment of coeliac patients is based on the maintenance of a gluten-free diet, which means the exclusion of wheat, barley, rye, and oats from the daily diet. The introduction of oats into the gluten-free diet has been a topic of debate in recent years due to reports on differences in toxicity among cultivars.

With the aim of developing non toxic oat varieties well adapted to the conditions of Andalusia (Spain) a collection of accessions has been assessed for both toxiciy and agronomic performance, and a breeding program has been initiated. The demonstration that the new oat lines now been developed are non-toxic and would be, therefore, suitable for consumption by the coeliac population, will establish the convenience of incorporating this trait in future oat breeding programs.

High-throughput metabolic profiling based on nuclear magnetic resonance (NMR) spectroscopy identifies signatures of heterotic pools and QTL for complex inherited traits in hybrid rye

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Winter rye (*Secale cereale* L.) is mainly bred as a hybrid crop in Germany. For the continuous improvement of grain yield, quality and sustainability in rye, a better understanding of the genetic basis of various physiological, developmental and morphological traits is required. Since the metabolome is a fundamentally important biochemical manifestation of a genome and combines a high level of condensed information, the availability of large-scale automated analytical platforms render the metablome a highly attractive target for functional genetic approaches. Nuclear magnetic resonance (NMR) spectroscopy provides a powerful, stand-alone, single analysis technique to identify and quantify the complete set of metabolites in a cell or tissue type without bias. We have evaluated the potential of NMR spectroscropy as a novel and innovative option for functional genetic approaches in rye. Here, we report on the establishment of standardized high-throughput protocols on sample preparation and acquisition of NMR spectra from rye leaves. We have comprehensively analyzed the metabolome of randomly selected individual plants from 37 open pollinated rye populations, including two populations representing both heterotic gene pools in rye, as well as 96 elite rye inbred lines. We have identified metabolic signatures for both heterotic gene pools in rye indicating, that NMR-based metabolic profiling is a powerful approach to efficiently search for genetic resources in rye, which are genetically diverse from the Petkus gene pool. Furthermore, NMR spectroscopy allowed us to establish metabolic profiles for a set of 197 and 320 extensively phenotyped experimental hybrids. We have identified metabolic features, which are significantly associated with individual QTL for agronomic traits in rye hybrids. These results demonstrate the potential of NMR spectroscopy to exploit large-scale metabolic information, which complements genomic approaches for the prediction of complex, polygenic traits in rye hybrids.

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Automated QTL mapping using high-throughput phenotypic data in wheat and barley

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Over the past few decades, considerable amount of useful genetic information generated by increasingly easier and cheaper approaches to sequence genomes and develop markers has enhanced the genetic knowledge of various crop species. In order to address issues global agriculture is facing, such as measures to ensure food security, information of this nature has to be properly linked to phenotypic data.

Phenotyping, however, is recognized as a laborious and technically challenging part of the process. In recent years, initiatives as The Plant Accelerator (TPA) in Adelaide have contributed to relieve these constraints by providing large scale phenotypic measurements through fully automated imaging and watering systems.

In this context, an R package has been developed to automate steps of data analysis at TPA, allowing proper linkage between genotypic and phenotypic datasets.

Five wheat mapping populations and four barley mapping populations have been used to identify genomic regions related to salinity tolerance by the Salt Focus Group at ACPFG. To investigate plants that maintain high growth rate despite external Na^+, all mapping populations have been imaging-based phenotyped at TPA under salinity and control conditions (osmotic datasets) as well as had some ionic concentrations measured (ionic datasets).

At this stage, the package allows identify and report unusual patterns as well as outliers for all high-throughput datasets tested (salinity or other non-salinity TPA experiments). It also combines correctly genotypic and salinity phenotypic datasets (osmotic or ionic) to generate inputs for QTL analysis. The outputs of Interval Mapping, Composite Interval Mapping and/or Permutation Tests are used to create appropriate graphics and tables. All relevant outputs, from outlier detection to QTL identification for both osmotic and ionic datasets are reported in the same pdf document for each mapping population. Preliminary results will be presented.

proWeizen – the German wheat research and breeding alliance

T Gerjets

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Wheat is one of the world's most important crops and Germany is among the ten most important wheat producers in the world. In recent years, yield improvements in wheat lagged behind those observed in other major crops. To overcome this, it is necessary to both join and increase efforts in wheat breeding and research.

In 2012, the German wheat breeders founded the German Wheat Research and Breeding Alliance proWeizen to combine the scientific excellence in wheat research and the breeding expertise in Germany. The new alliance is a public-private partnership to foster wheat breeding and research in Germany and internationally as well as a platform for communication and coordination. The proWeizen platform is equally open to scientists and companies working in wheat breeding and research.

proWeizen liaises between wheat breeding and wheat research on national and international level, participates in national and international efforts of wheat research and breeding and helps with mobilizing of funding opportunities.

A TALEN platform for plant research and biotechnology

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Transcription activator-like effector nucleases (TALENs) are customizable fusion proteins able to cleave virtually any genomic DNA sequence of choice *in situ*, and thereby to generate site-directed genetic modifications in a wide range of experimental cell systems and whole organisms. TALEN-mediated manipulation of genes, involving either their knock-out or replacement, is an attractive technology for both basic research and plant breeding.

To be prepared for the growing demand for customizable endonucleases for monocot and dicot plants, we are about to establish a technological platform involving TALEN design, vector construction, cleavage-activity testing, TALEN expression, mutant detection and generation of homozygous mutant lines. Upon web-based design of TALE-based DNA binding domains including *in silico* analysis for off-targets in the host genome, TALEN coding sequences can be assembled *via* 'Golden Gate' cloning using plasmid libraries available to the scientific community (www.addgene.org/TALEN/guide). The constructs for the two TALEN- units required for cleavage activity can be cloned either into separate or double cassette transformation vectors, or coupled in a chimeric gene by a viral ribosomal skipping signal (e.g. T2A) that facilitates the translation of the TALEN units into two autonomous proteins. To streamline the production of transformation vectors, we have developed generic plasmids that enable us to readily integrate any target gene-specific TALEN units into monocot and dicot-compatible expression cassettes that can then be transferred to a choice of acceptor binary vectors carrying various plant selectable marker genes. Transient or stable TALEN expression is performed using transformation methods available for quite an array of model and crop plants. To produce non-chimeric, homozygous mutants, genetic transformation is ideally coupled with or followed by the use of haploid technology which is well deployed in the lab as well. Altogether, this integrative approach will greatly facilitate the further development of TALE-based methods and contribute to a broad application of this breakthrough technology in plant research and crop improvement.

Potential of biogas production by using winter pea mixtures with triticale and oat

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Production of methane-rich biogas through anaerobic digestion of organic materials has been evaluated as one of the most energy efficient and environmentally benign pathways of renewable energy generation. Many conventional forage crops are easy to cultivate and produce large amount of biomass. Furthermore, being bred for animal feed these crops are often characterized by good digestibility. Traditionally in the Republic of Serbia mixtures of winter pea with winter cereals are used extensively for forage production as the first crops in double cropping system which involved as the second crops maize and sorgho for silage.

The main purpose of this study was to evaluate the feasibility of biogas and methane production from sole crops of winter pea, triticale and out and their mixtures at two different maturity stage (1.full-flowering stage of winter pea, 2. milky-waxy stage of cereals). A field experiment was conducting during the 2012-2013 growing season at the experimental field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Winter pea (*Pisum arvense* L.), oat (*Avena sativa* L.) and triticale (× *Triticosecale*) monocultures as well as mixtures of winter pea with both of cereals, in two seeding ratios (50:50 and 75:25) were used to investigate: forage yield (t ha^{-1}), dry matter yield (t ha^{-1}), yield of organic solids (t ha^{-1}) and calculated parameters of biogas and methane production (biogas yield from dry matter (m^3 ha^{-1}), biogas yield from organic solids (m^3 ha^{-1}), methane yield from dry matter and methane yield from organic solids (m^3 ha^{-1})).

All samples were investigated regarding potential biogas yield and methane content, in batch equipment with 1 L glass bottles in climate chamber. Temperature mode was mesophilic (38 °C) and the samples were inoculated with the digestate from operating biogas plant in the vicinity, which as substrates use cattle manure and maize silage. Highest dry matter yield, yield of organic solids and calculated parameters of biogas and methane production, except yield of organic solids, was achieved with triticale monoculture in the second stage of investigation (milky-waxy stage of cereals). The results showed that mixtures of winter pea with both of cereals at the seeding ratio (75:25) achieved a higher methane yield than the other mixtures studied.

A non-destructive method to assess changes in the biomass of small-grain cereals under field conditions

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This study describes a method to estimate the pattern of changes in crop dry weight (CDW) of triticale and oats on the basis of spectral reflectance measurements. Field experiments involving 30 cultivars of triticale and 30 cultivars of oats were conducted in northeastern Spain in 2011 under irrigated conditions, with a total water input of 410 mm. Reflectance spectra were captured on five occasions from the beginning of jointing to anthesis, and reflectance values at 680 nm and 771 nm were used to calculate the normalized difference vegetation index (NDVI). A destructive sample was taken on each plot at the early dough stage of grain development to determine aboveground biomass production. Triticale cv. 'Algoso' (21,357 kg DM ha^{-1}) and the oats cv. 'CHD2316/03' (21,007 kg DM ha^{-1}) gave the highest biomass production. Daily biomass increments were inferred from NDVI values and from radiation data obtained from a meteorological station located close to the experimental fields. On average, biomass production and radiation use efficiency (RUE) were greater in triticale than in oats, but oat cultivars showed larger genetic diversity in both traits.

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