Proceedings of the 1st Workshop on TritiGen COST action FA0604:

Triticeae genomics for the advancement of essential European crops

October 1-3, 2007
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1st Workshop on TritiGen COST action FA0604: Triticeae genomics for the advancement of essential European crops

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SCIENTIFIC PROGRAM

October 1-3, 2007
Semiramis Hotel, Puerto de la Cruz, Tenerife

MONDAY, October 1

8:00-8:45: Registration and poster mounting

8:45-9:00: Welcome

***Session 1: WG1: Tools for Assessing and Harvesting Genetic Diversity* (DivGen)**

Chairs: Karl Schmid and Hikmet Budak

09:00-09:20 Marco Maccaferri, Italy.
A durum wheat germplasm collection evaluated for adaptation to drought-prone Mediterranean environments and used in an association mapping approach

09:20-09:40 Andy Flavell, UK.
Genomics-assisted analysis and exploitation of barley diversity - the EXBARDIV ERA-PG Project

09:40-10:00 Etienne Paux, France
Insertion site-based polymorphism: recycling the 'junk' from the wheat genome

10:00-10:20 Ana Casas, Spain
Linkage disequilibrium in the Spanish Barley Core Collection

10:20-10:40 Agostino Fricano, Italy
BARLEYSEL: Assessing domestication effects on candidate genes implicated in controlling frost resistance and malting quality

10:40-11:00 Viktor Korzun, Germany
Molecular markers and their applications in cereal breeding - from breeders point of view

11:00-11:30 Coffee Break and poster viewing

11:30-11:50 Hikmet Budak, Turkey
Organelle genome variation and transient gene expression in the model grass species *Brachypodium distachyon*, accurate template for wheat and barley

11:50-12:10 András Bálint, Hungary
Mapping of QTLs for drought tolerance in barley at different developmental stages

12:10-12:30 Ahmet Yildirim, Turkey
Genetic variations are still continuting among and between landraces of wheat in Turkey

12:30-12:50 Karl Schmid, Germany
Allele mining in wild and cultivated barley

12:50-13:00 General discussion

13:00-14:00 Lunch (Hotel Semiramis Restaurant)

Session 2: WG2: Accessing the Physical Genome for Sustainability and Quality (PhysGen)

Chairs: Pierre Sourdille and Nils Stein

14:00-14.:20 Daniela Schulte, Germany
Towards physical mapping of the barley (*Hordeum vulgare*) genome

14:20-14:40 Robert Kofler, Austria
Development and physical (bin) mapping of SSR markers for the short arm of rye chromosome 1

14:40-15:00 Katerina Pankova, Czec Republic
Genetic, physiological and molecular analyses of wheat lines carrying flowering time genes on chromosome 3B substituted from the alternative wheat variety Ceska Presivka

15:00-15:20 Nikolai Christov, Bulgaria
Identification of cDNAs involved in winter wheat cold acclimation process

15:20-15:40 Tzion Fahima, Israel
Unraveling the genetic basis of several qualitative and quantitative agronomic traits derived from wild emmer wheat

15:40-16:00 Nicola Pecchioni, Italy
Identification and characterization of conserved orthologous markers between barley and *Brachypodium*

16:00-16:30 Coffee break and poster viewing

16:30-16:50 Jaroslav Dolezel, Czec Republic
Production of subgenomic BAC libraries and other recent activities related to chromosome sorting in cereals

16:50-17:10 Kostya Kanyuka, UK
Towards fine mapping of resistance genes to *Septoria tritici* leaf blotch in hexaploid and diploid wheat

17:10-17:30 Hélène Bergès, France
How to preserve, maintain and exploit plant genomic resources generated by international research.

17:30-17:50 General discussion

Session 3: WG 2A Bioinformatics for WG1 and WG2

Chair: David Marshall

17:50-18:10 Tatjana Sjakste, Latvia
Functional significance of the structural rearrangements of candidate genes

18:10-18:30 François Sabot, Finland
Handle the junk - A unified classification system for eukaryotic TEs

18:30-18:50 Thomas Wicker, Switzerland
Sequencing a large genomic region in wheat

18:50-19:00 General discussion

20:30 Workshop Dinner (Magnolia Restaurant)

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Session 4: WG3: Implementation of Genomics Approaches for Understanding Cereal Traits

Chairs: Nils Rostoks and Hilde-Gunn Opsahl- Sorteberg

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Genes regulating seed development.

09:20-09:40 Laura Rossini, Italy
Candidate gene and functional genomics approaches for the molecular dissection of barley development

09:40-10:00 Anette Moldestad, Norway
Changes in the proteome pattern during grain development of cereals

10:00-10:20 Christine Finnie, Denmark

Functional proteomics of barley seeds: Integration of the proteome with the genetic map, gene expression and enzyme activities.

10:20-10:40 Bill Thomas, UK
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10:40-11:00 Gabor Galiba, Hungary
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11:30-11:50 Roberto Tuberosa, Italy
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Genomic Dissection of Drought Resistance in Wild Emmer Wheat

12:30-12:50 Barbara Steiner, Austria
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Functional analysis of antifungal activities in a wheat multidomain cystatin TaMDC1

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16:50-17:10 Huw D Jones, UK
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17:10-17:30 Andy Phillips, UK
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17:30-17:50 Valentina Talamè, Italy
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Chair: Thomas Wicker

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18:20-18:40 Christos Ouzounis, Greece
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18:40-19:00 Doreen Ware, UK
Mathematical repeats and their uses for annotating plant genomes

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Chairs: Alan Schulman and Catherine Feuillet

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Session 3: WG 2A Bioinformatics for WG1 and WG2

Session 5: WG 4 Functional Genomics for Testing and Validation of Candidate Genes (FuncGen)

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Session 1: WG1: Tools for Assessing and Harvesting Genetic Diversity (DivGen)

S1

A DURUM WHEAT GERMPLASM COLLECTION EVALUATED FOR ADAPTATION TO DROUGHT-PRONE MEDITERRANEAN ENVIRONMENTS AND USED IN AN ASSOCIATION MAPPING APPROACH

Maccaferri M.[1], Sanguineti M[1], Natoli V.[1], Araus Ortega J.[2], Ben Salem M [3], Bort J.[2], De Ambrogio E.[4], Garcia del Moral L.[5], Martos V.[5], De Montis A.[4], El-Ahmed A.[6], Elouafi I.[7], Maalouf F.[8], Machlab H.[8], Moragues M.[9], Nachit M.[7], Nserallah N.[10], Ouabbou H.[10], Royo C.[9], Tuberosa R.[1]*

[1] Dept.of Agroenvironmental Science and Technology, University of Bologna, Bologna, Italy, [2] Departament de Biologia Vegetal, Universitat de Barcelona, Barcelona, Spain ,[3] Tunisian National Institute of Agronomic Research, Tunis, Tunisia, [4] Società Produttori Sementi Bologna, Divisione Ricerca, Argelato (BO), Italy, [5] Dpto. Fisiologia Vegetal, University of Granada, Granada, Spain, [6] Plant Protection Department, Aleppo University, Aleppo, Syria, [7] ICARDA, Aleppo, Syria,[8] Department of Plant Breeding, Lebanese Agricultural Research Institute, Bekaa, Lebanon, [9] Area de Conreus Extensius Centre Udl-IRTA, Lleida, Spain and [10] CRRA-INRA, Settat, Morocco

** Corresponding author: E-mail: roberto.tuberosa@unibo.it*
Prof. Roberto Tuberosa

Germplasm collections of adapted materials held useful alleles for yield, yield stability and its components and the underlying chromosome regions can be identified using association mapping studies. A germplasm collection of 189 elite durum wheat accessions was selected from several Mediterranean countries as well as from the CIMMYT and ICARDA breeding programs.
Sixteen field trials were carried out in 2004 and 2005 in Italy, Spain, Morocco, Tunisia, Syria and Lebanon, including three check cultivars.
Across the environments, the materials showed an average yield ranging from 0.9 to 8.3 ton/ha. Yield, yield components, agronomic and physiological traits were recorded and tested for significant associations with the allelic profile of 186 SSR markers distributed over the 14 linkage groups. Mapped SSR loci were used to evaluate the population structure of the collection and were tested for association with the range of traits recorded in the field trials. Population structure as evaluated in STRUCTURE showed the presence of significant subgrouping, with 5 main subgroups. In general, significant LD among loci was found within 10 cM a part.
For each of the traits characterized by the highest heritability values among those measured, i.e. plant height, heading date, peduncle length and thousand kernel weight, it was possible to identify SSR markers showing significant associations in four up to 8/10 environments, with R^2 values ranging from 5 to 10 %. As to yield and yield components, the majority of the markers could be identified only in one to four env.s, with average R^2 values lower than 5%. The results will be compared with the QTLs found for the same traits in a mapping population evaluated within the same project and with those published in wheat up to now.

S2

GENOMICS-ASSISTED ANALYSIS AND EXPLOITATION OF BARLEY DIVERSITY - THE ERA-PG EXBARDIV PROJECT

Flavell A.[1], Pillen K.[2], Schulman A.[3], Graner A.[4], Cattivelli L.[5], Rasmussen S.[6], Russell J.[7]

[1] University of Dundee at Scri, [2] Max Planck Institute, Koln, [3] Mtt Helsinki, [4] IPK Gatersleben, [5] Cra - Experimental Institute for Cereal Research, Fiorenzuola d'Arda, [6] Copenhagen University, [7] Scottish Crop

Crop plants have evolved from their wild ancestors during domestication and selective breeding over the last ca. 10 000 years. First, wild plants carrying promising traits were cultivated, leading eventually to locally adapted landraces. Modern breeding has taken a limited selection of this germplasm and crossed the 'best with the best' to yield modern cultivars. Unfortunately, there are indications that we are approaching a performance ceiling for at least some crops, as the best alleles available become combined together. A potential escape route from this cul-de-sac is provided by introducing fresh alleles into cultivated materials from wild and old, locally adapted germplasm. This approach has already been successful in introducing resistance genes from the wild into cultivars. The challenge for future molecular breeding is to streamline this process, using high throughput genomics approaches, to handle less tractable but equally important traits affecting yield and adaptability. One particularly promising approach is association analysis, which compares genotype and phenotype data for heterogeneous populations and looks for links between these two parameters. This approach is intrinsically more powerful than 'classical' genetic linkage mapping because it scrutinises the results of thousands of generations of recombination and selection. However, association mapping faces a paradox - it is relatively easy to detect marker-trait associations in highly inbred populations, such as modern cultivars, but this inevitably results in a low resolution map, requiring more work to pin down the gene allele responsible for the trait. Conversely, highly diverse populations provide high-resolution associations but the numbers of markers needed to find any association are extremely high. Nevertheless, association mapping has been fruitful in human genetic studies and is just beginning to be tested in plants. Barley is an ideal prototype for such a study, largely because thousands of gene-based SNP polymorphisms are available. The goal of the EXBARDIV project is to apply these high throughput markers to European barley cultivars and combine these data with detailed phenotypic analysis to identify new associations. These promising 'low resolution' associations will be refined to yield more accurate associations by repeating the study in locally adapted barley landraces and, if necessary, a final high resolution association analysis will be performed in a wild barley collection to pin down the exact gene alleles responsible for the traits. The EXBARDIV project, which begins October 2007, brings together 7 European research institutions with extensive experience in barley genome and phenotype analysis. The talk will summarise the proposed approaches to be used in the project and the germplasm that has been selected for study.

S3

INSERTION SITE-BASED POLYMORPHISM: RECYCLING THE 'JUNK' FROM THE WHEAT GENOME

Paux E.[1], Roger D.[1], Chevalier K.[1], Paux K.[1], Cakir M.[2], Gandon B.[3], David J.[1], Bernard M.[1], Sourdille P.[1], Feuillet C.[1]

[1] Inra, [2] State Agricultural Biotechnology Center, [3] Limagrain Verneuil Holding

Transposable, elements (TEs) are prevalent in most plant genomes. They are ubiquitous, in high copy numbers, evenly distributed in the genome, in both hetero- and euchromatin, and show insertional polymorphism both within and between species. In wheat, TEs account for more than 80% of the genome. From a quantitative perspective, it is easy to see that they are the most significant factors in determining the structure of this genome. It is likely that TEs have driven wheat genome evolution in diverse ways, including genome expansion and contraction, segmental duplication, and exon shuffling. It has been proposed that TE-induced genomic rearrangements tend to promote both cytological and genetic diploidization of the hexaploid genome. Therefore, TE-based molecular markers represent ideal tools to study the structure and evolution of the hexaploid wheat genome. In the framework of a BAC-end sequencing project, we have recently demonstrated the potential of small genomic sequences from wheat for developing new TE-based molecular markers. This method, called Insertion Site-Based Polymorphism (ISBP), exploits knowledge of the sequence flanking a TE, and uses one primer designed in the TE and the other in the flanking DNA sequence. We have developed several hundreds of ISBP markers that are representative of all kind of junctions (various TE families in both repetitive and low copy DNA, either coding or non-coding) and are evenly distributed along chromosome 3B of bread wheat. Various detection techniques have been validated including agarose and acrylamide gel electrophoresis, melting curve analysis and temperature gradient capillary electrophoresis. More recently, sequencing on diploid, tetraploid and hexaploid wheat has revealed a higher level of polymorphism in ISBP sequences with both SNPs and IDPs. Thus, ISBP represent a new source of polymorphism in wheat, allowing the development of several thousands of markers evenly distributed along the chromosomes. Examples of their applications including cytogenetic, genetic and physical mapping, evolution and phylogenetic studies, recombination and linkage disequilibrium analyses as well as marker-assisted selection will be presented.

S4

LINKAGE DISEQUILIBRIUM IN THE SPANISH BARLEY CORE COLLECTION

Casas A., Casao C., Yahiaoiu S., Gracia M., Lasa J., Igartua E.

EEAD-CSIC, Zaragoza, Spain

The Spanish Barley Core Collection (SBCC) is a representative sample of inbreds derived from Spanish landraces, collected prior to the introduction of modern cultivars. It has good potential for gene or allele discovery both for abiotic and biotic stress resistance. The SBCC is mainly composed of two large populations of individuals. Most likely, these populations correspond to the entry of two different sets of barley ancestors in the Iberian peninsula, ensued by only partial admixture. Their distribution across the range of local climates indicates differential adaptation of these populations to prevailing environmental factors. The objectives of this study are i) to determine the extent of linkage disequilibrium (LD) in the SBCC, in order to assess the feasibility of association mapping, and ii) to search for selection signatures by looking at genome scans of allelic diversity, LD, population differentiation, and geographic distribution of diversity. The SBCC was examined with DArTs. From a total of over 850 polymorphic markers, 732 presented good quality and known map position. Coverage of chromosomes was rather uniform, except for chromosome 4H. LD in this collection is clearly noticeable up to 2-3 cM. This makes it suitable for attempting association mapping, as enough genome coverage can be achieved by using affordable marker technology currently available for the species. The distribution of polymorphism information content (PIC), LD, and population differentiation (measured as Fst) across the genome is not uniform. Some chromosomal regions presented unusual profiles for several of these parameters at the same time. We hypothesize that these regions may harbour genes that favored adaptation of Spanish barley populations to their home environments.

S5

BARLEYSEL: ASSESSING DOMESTICATION EFFECTS ON CANDIDATE GENES IMPLICATED IN CONTROLLING FROST RESISTANCE AND MALTING QUALITY

Fricano A.[1], Baldassarre V.[2], Faccioli P.[2], Stella A.[1], Rizza F.[2], Gianinetti A.[2], Cattivelli L.[2], Rossini L.[3], Pozzi C.[1], Salamini F.[1], Piffanelli P.[1]

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Scientific evidences of archaeological remains has revealed that the origin of western agriculture occurred about 10,000 years ago in a region of the Middle East known as the Fertile Crescent, where the wild progenitors of several key agricultural cereal species are endemic. Domestication of cereals entailed the appearance of agronomic traits such as seed size and resistance to abiotic stress. For a representative collection of 172 domesticated barley (*Hordeum vulgare*) lines and 66 *Hordeum spontaneum* genotypes we determined the haplotype profiles of 5 candidate genes implicated in the control of frost tolerance and malting quality. We used high-throughput technologies available at the PTP Genomics Platform to create a biorepository of the germplasm collection and carry out automated AFLP analysis and SNP identification. In parallel, the BARLEYSEL collection was phenotyped for both frost tolerance and malting quality to undertake correlation studies genotype-phenotype. Bioinformatics analyses are underway to determine the existence of domestication effects on the analysed candidate genes to unravel the evolutionary history of cultivated barley and to quantify its impact on genetic diversity.

S6

MOLECULAR MARKERS AND THEIR APPLICATIONS IN CEREAL BREEDING - FROM BREEDERS POINT OF VIEW

Korzun V.

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The size and structure of cereal genomes (in particular barley, rye and wheat) make them one of the most complex series of crop species for genetic analysis and has resulted in difficulties in applying this information to genetics and practical breeding. Nevertheless, the development of molecular techniques for genetic analysis has led to a great increase in our knowledge of cereal genetics and our understanding of the structure and behaviour of cereal genomes. These molecular techniques, in particular the use of molecular markers, have been used to monitor DNA sequence variation in and among the species and create new sources of genetic variation by introducing new and favourable traits from landraces and related grass species. Improvements in marker detection systems and in the techniques used to identify markers linked to useful traits, has enabled great advances to be made in recent years. Identification of markers linked to useful traits has been based on complete linkage maps and bulked segregant analysis. However, alternative methods, such as the construction of partial maps and combination of pedigree and marker information, have also proved useful in identifying marker/trait associations.

Conventional cereal breeding is time consuming and depends on environmental conditions. Breeding a new variety takes between eight and twelve years and even then the release of an improved variety cannot be guaranteed. Hence, breeders are extremely interested in new technologies that could make this procedure more efficient. Molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improve the selection strategies in cereal breeding. The value of markers in analysing the inheritance of traits in crop plants and understanding genome structure and organization is now well established. The promise of marker-assisted selection in crop breeding still remains but achieving practical benefits takes longer than expected. The main reasons for this delay are the insufficient quality of markers (regarding their predictive and/or diagnostic value), inadequate experimental design, high costs and complexity of quantitative traits. Only close interactions between breeders and biotechnologists will accelerate the effective implementation of marker-assisted selection in cereal breeding programmes.

A revision of current breeding methods by utilizing molecular markers in breeding programmes is, therefore, crucial in this phase. Molecular techniques and their application to genome analysis and molecular breeding of cereals species will be discussed.

S7

ORGANELLE GENOME VARIATION AND TRANSIENT GENE EXPRESSION IN THE MODEL GRASS SPECIES BRACHYPODIUM DISTACHYON, ACCURATE TEMPLATE FOR WHEAT AND BARLEY

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The improvements in molecular biology and genetics studies depend highly on the availability of a suitable model organism. *Brachypodium distachyon* with its physical and biological characteristics has proposed to be a model species for the grass family, Poaceae, that includes major cereal crops such as wheat and barley. In this research, the organelle genome diversity and relationships of 1000 genontypes representing a representing diverse geographic regions were evaluated using chloroplast (cp) and mitochondrial (mt) DNA PCR-RFLPs. MtDNA variation indicated that the number of polymorphic loci (24) was low and genetic differentiation was Gst=0.60, excluding the outgroups [hexaploid wheat (*Triticum aestivum*) and triticale (*X Triticosecale* Wittmack)]. CpDNA analysis revealed low level of polymorphism (35%) among the accession and Gst was 0.39. One cpDNA and two mtDNA fragment bands were significantly correlated to the site of germplasm region; although there was no clear trend. Under various combinations of bombardment pressures and sample plate distances using gold particles of different diameters, the efficiency of gene delivery in *Brachypodium* was evaluated by assessing the transient GUS expression on bombarded tissues. Selection and regeneration of the pututative transformants in optimizing the regeneration conditions were also evaluated. The results indicated that the level of organelle polymorphism is low among the genotypes used in this study and transient GUS gene expression in *Brachypodium* accessions was highly efficient.

S8

MAPPING OF QTLS FOR DROUGHT TOLERANCE IN BARLEY AT DIFFERENT DEVELOPMENTAL STAGES

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Drought is one of the most important abiotic stresses, which causes yield loss of cultivated plants. Determining major QTLs influencing drought tolerance could be helpful for identifying drought tolerant genotypes. The aim of our work reported here was the finding major QTLs for drought tolerance in barley at different developmental stages and testing under different environmental conditions. Double haploid (DH) lines of the Oregon Wolfe Barley (OWB) population were screened for drought tolerance. The OWB population is saturated with Expressed Sequence Tags (ESTs), which could be useful to understand the basic mechanism of drought tolerance (functional mapping). In order to determine QTLs involved in PEG-induced osmotic stress tolerance in barley, the Oregon Wolfe Barley (OWB) mapping population was examined at the germination and seedling stage. To find QTLs influencing post anthesis drought tolerance (PADT) the same population was screened for drought tolerance in greenhouse, growth chambers and in field. Drought tolerance at germination stage was determined based on the reduction on the shoot and root lengths, while at seedling stage the drought stress caused reduction in the shoot dry weights were used. For the mature plants the drought tolerance was calculated from the yield loss and 1000 grain weight reduction caused by the stress. In the greenhouse experiment the QTLs affecting the relative water contents and osmotic adjustment were also mapped. QTL analysis was performed for all investigated characters. The results revealed that the most effective QTLs for drought tolerance in seedling-, young plant- and mature stage are different; however, common QTLs with lower effect were also determined in each developmental phase. Some QTLs identified at the germination stage may be not specific for drought, because the same QTLs were determined for salt tolerance at the germination stage and for pre-harvest sprouting and dormancy. Therefore, these QTLs seem not to be specific for drought-, but rather for general abiotic stress tolerance.

S9

GENETIC VARIATIONS ARE STILL CONTINUING AMONG AND WITHIN THE LAND RACES OF WHEAT IN TURKEY

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Genetic characterization of landraces which are one of the most important gene sources in wheat breeding facilitates their usages in breeding. Morphological, protein and molecular markers such as DNA markers have been used to characterize plant genomes. In recent years, mostly microsatellite markers (SSRs) have been chosen in molecular characterization of wheat due to their advantages over other marker systems. In this study, molecular characterization of 20 landraces of bread and durum wheats collected from different regions of Turkey was aimed. Several SSR primers were screened and thirteen most polymorphic primers in durum wheat and ten primers in bread wheat were employed in characterization. DNA of 10 accessions from each landraces (totally 400 accessions) were isolated and specific DNA regions were amplified by PCR using each SSR primer. PCR products were run in 8 % Polyacrylamide gels. Polymorphism evaluations were done by naked eye as well as by using Vilber Lourmat, Bio 1D 11.04 software. Dendrograms of data were drawn based on band sizes of the PCR products. Results indicated that genetic variations among and within Turkish wheat land races were still exist. Morphologically similar genotypes were determined as genetically different based on SSR screenings. Dendrogram of the bread wheat land races were grouped into eight sub-groups while dendrogram of the durum wheat land races were grouped into two main sections, and these main sections were separated into several sub-groups. Number of alleles were between four and nine for the bread wheat land races whereas it changed between seven and eleven for the durum wheat land races. These results indicate that SSR markers could be successfully used in genetic characterization of durum and bread wheat landraces.

S10

ALLELE MINING IN WILD AND CULTIVATED BARLEY

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The utilization of genetic resources from wild relatives and ancestors of modern crop plants is becoming an important research paradigm in plant genetics. The rapid development of genomic technologies will be very useful in reaching such a goal. We have begun a research program to characterize naturally occurring genetic variation in wild and cultivated barley. Wild barley occurs throughout ecologically diverse habitats and has been shown to be locally adapted to different environments. Our goal is to investigate those adaptations and to identify genes responsible for it.
We have collected wild barley from various sites in Israel, particular from a microsite called the 'Evolution Canyon' which is characterized by a strong environmental gradient within few hundred meters. A total of 56 accessions were sequenced at 37 randomly chosen EST-derived loci and standard measures of population genetic variation were calculated to evaluate genome-wide patterns of variation. The distribution of summary statistics is similarly to the predominately self-fertilizing species Arabidopsis thaliana. There is also an extensive geographic population structure across Israel. In particular, accessions from the two slopes of the Evolution Canyon (*Mesic versus xeric*) show a strong pattern of genetic differentiation.
We also sequenced candidate genes possibly involved in local adapation from a sample of wild barley accessions and a core collection of cultivated barley varieties. They include genes controlling flowering time and disease resistance. On average, these genes tend show a lower level of genetic variation in cultivated than in wild barley, which is consistent with a bottleneck during inbreeding and subsequent inbreeding of modern cultivars. We will discuss the implications of our results for the utilization of genetic variation in barley.

Poster abstracts

Session 1: WG1: Tools for Assessing and Harvesting Genetic Diversity (DivGen)

P1.1

THE MOLECULAR DIVERSITY OF BULGARIAN CEREAL GERMPLASM COLLECTIONS – A REFERENCE POINT FOR BETTER UNDERSTANDING, EXPLOITATION AND BROADENING OF THE GENETIC BASE OF CEREAL CROPS

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Cereals (bread and durum wheat, and barley) constitute about 50% of the crop production in Bulgaria and are the most important renewable resources for food, feed and industrial raw material. This make the region's economy largely dependant on cereal production. Knowledge of genetic diversity in cereal germplasm helps to ensure that a broad genetic base of breeding materials is maintained not just for sustaining genetic improvement but also for reducing genetic vulnerability to pest, biotic and abiotic stresses. In this study the genetic diversity in Bulgarian wheat (*T. aestivum* L. *and T. durum* Desf.) and barley (*H. vulgare* L.) germplasm collections was evaluated and compared to those of Belgian and Cypriot collections using genomic- and EST-SSR markers. Bulgarian bread wheat genetic diversity showed no overall reduction over the last 60 years. The estimates of genetic variability in modern Bulgarian and Belgian bread wheat cultivars and advanced breeding lines clearly revealed that the Bulgarian germplasm is more diverse. The genetic diversity was unequally distributed among the seven homeologous groups and the three genomes of wheat. The observed pattern of chromosome and genome specific diversity was different in Bulgarian and Belgian wheat gene bank collections. Bulgarian durum wheat cultivars released since 1975 were substantially more diverse than the present-day germplasm from Cypriot gene bank collection. Numerous unique alleles, that are absent in modern cultivars, were found in old Bulgarian and Cypriot durum wheat cultivars and landraces. The latest represent valuable source for broadening the genetic base of durum wheat breeding. As opposed to wheat results, the barley cultivars released since 1980 and representing the most relevant Bulgarian and Central European germplasm showed considerably lower level of genetic variability than the elite cultivars from Cypriot gene bank collection. The UPGMA Cluster analysis was performed for bread and durum wheats as well as barley. In all cases Bulgarian, Belgian and Cypriot accessions were separated in distinct clusters and subclusters. The cluster distribution was generally in accordance with the agro-geographical site of origin of the collections and the level of genetic diversity. The results of this study illustrate the impact of Bulgarian, Cypriot and Belgian breeding programs on important crop species such as wheat and barley and provide useful information for further improvement of cereals.

Acknowledgements: This work was partially supported by the International Atomic Energy Agency (Research Contract No RER5013), the National Scientific Program "Genomics" (project No G-5-01/2003), Bulgarian MES (grant No CC1515/05) and Bulgarian-Belgian bilateral project (project No 10, FC/BUL-2006/00386).

P1.2

NS-GENOME SPECIFIC DNA SEQUENCES FROM LEYMUS

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The Ns-genome specific DNA sequences have been isolated from two *Leymus* species: L. mollis and L. arenarius. Five out of six clones characterized, i.e. pLmIs1, pLmIs44, pLmIs51, pLmIs53 and pLaIs56, are dispersed retrotransposon-like repeats, and one (pLaIs7) is a chloroplast gene. These sequences are highly specific to *Leymus* and *Psathyrostachys* as they hybridize essentially to these species, while little or no signal can be detected in other *Triticeae* species. Fluorescence in situ hybridization (FISH) mapping of these sequences shows that they are dispersed indiscriminately over all chromosomes. Southern hybridization experiments using total genomic DNA as probes confirm that there is nothing else in the *Leymus* genomes but Ns-genomic DNA. Based on these evidence, Leymus must be considered autopolyploid having the (Ns)n genome designation or segmental allopolyploid consisting of a variation of the basic Ns-genome. To examine further how species in these two genera are related, genetically and genomically, and to what extent the basic Ns-genome has distributed in the *Triticeae*, we have included in the study more than 30 species and accessions of known, or suspected, to have the Ns-genome. Restriction fragment length polymorphisms (RFLPs) generated by these Ns-genome specific sequences are used to construct phylogenetic trees, and as expected the Ns-genome is clearly differentiated from all other genomes examined. This Ns-genome cluster includes all of the *Leymus-Psathyrostachys* species, except two accessions that have been misidentified. Species in the genera *Hordelymus* and *Hystrix* also have the *Leymus* Ns-genome. Furthermore, the Ns-genome cluster appears to divide into two major groups: one containing all Eurasian *Leymus* species and the other comprising the rest, i.e. N-American and Asiatic *Leymus*, all species of *Psathyrostachys*, and other Ns-genome species. This separation is also in good agreement with taxonomic treatment within *Leymus*.

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P1.3

GENETIC DIVERSITY IN BARLEY MUTANT COLLECTION OF VILNIUS UNIVERSITY

Rancelis V., Vaitkuniene V., Balciuniene L., Cesniene T., Naugzemys D., Kleizaite V., Bieliuniene, A., Zvingila D.

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DNA polymorphism was assessed in barley mutant collection of Vilnius university using molecular markers. Among barley mutants there are some interesting developmental and homeotic mutants. Barley mutants tweaky spike (tw) were induced by chemical mutagens in barley cv. 'Auksiniai'. Mutation causes ectopic conversion of lodicules to stamens and /or carpels. Genetic instability was the one of peculiarities of some tw mutants. Reversions to normal type tw arised with rather high frequency (Rancelis et al., 2004). Some of these revertants (Rvs) differ in their morphological and agronomical characters. In the first series of investigations our attention was focused on the traits that are exclusive for tw type mutants. In the second part of investigations our attention was directed to the characteristics having an economic value, such as productivity and quality of seed production, resistance to diseases and lodging. Diversity of Rvs in a wide range of traits was observed in both parts of investigations and independently of the source of Rvs – from tw1 or tw2. Polymorphism in RAPD spectra was established among some studied revertants and tw plants. References Ranč lis V, Vaitk□nien□ V, Bal□i□ ien□ L, Ma□kinait□ R, Leistrumait□ A. Genetic variation for plant breeding 2004; Eds J. Vollman, H. Grausgruber and P. Ruckenbauer. EUCARPIA and BOKU, Vienna. 219-222.

P1.4

IDENTIFICATION AND CHARACTERIZATION OF NOVEL GLU-1DY LOCI ALLELE IN HEXAPLOID WHEAT (TRITICUM AESTIVUM L.)

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The wheat (*Triticum aestivum L.*) contains six genes encoding high molecular weight (HMW) subunits of glutenin. Each of the loci Glu-1A, Glu-1B and Glu-1D contains tightly linked the genes x and y, which are related but have subtle differences in their structures and properties. We analyze old wheat varieties and landraces of the Carpathian Basin in Central Europe and we found one new allele which is encoded by Glu-1D loci. Landraces Noe was identified as having a subunit with unusual electrophoretic mobility in SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) We confirm our finding by size-exclusion high-performance liquid chromatography (SE-HPLC) and also by reversed-phase high-performance liquid chromatography (RP-HPLC). The new HMW gene Glu-1D12.3, complete cds was determined by primer walking on overlapping nested deletions and deposited under accession number (GeneBank EF472958).

P1.5

SELECTION AND EVALUATION OF A REPRESENTATIVE SET OF WILD BARLEY INTROGRESSION LINES (ILS)

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Introgression lines (ILs) contain small chromosomal segments from a donor accession which are present in the genetic background of a recipient line (Zamir 2001). A complete set of overlapping exotic introgression lines will represent the entirety of an exotic genome. These ILs can be used for instance to identify and verify QTLs. In barley, we are selecting a representative set of overlapping wild barley introgression lines starting from 40 BC2DH lines of the spring barley population S42 (von Korff et al. 2004). Based on selection with SSR markers, we have so far assorted 53 S42-ILs containing single introgressions from the wild barley (*Hordeum vulgare* ssp. *spontaneum*) donor accession ISR42-8 present in the elite background of the spring barley cultivar Scarlett (Schmalenbach & Pillen, 2007). At present, 70.3 % of the exotic barley genome is already covered by the selected ILs. These lines contain an average exotic introgression of 31.7 cM with a range from 7.0 - 100.5 cM. Currently, the selected ILs are subjected to high resolution genotyping with DArT (Diversity Arrays Technology) markers in order to precisely characterize the extent of each introgression. In 2007, the selected ILs are tested in multi-field experiments and under controlled conditions in climate chambers. Applying a general linear model to the field data using S42-ILs as the source of variation and the recurrent parent Scarlett as the control, we could verify previously located QTLs for agronomic traits like flowering time, plant height, yield, pathogen resistance and others. In future, promising QTL effects which are verified in S42-ILs will be subjected to map-based cloning projects. In addition, the marker resolution of the barley IL set will be further increased by means of the Illumina 1.5 k barley SNP chip. On the other hand, the size of the introgressions will be improved by selecting so called Sub-ILs containing small introgressions below 5 cM. The advantages of the wild barley ILs are manifold. (1) The wide cross carried out to generate the S42-ILs increases the chance of finding contrasting allelic effects which is a prerequisite to detect QTLs. (2) The QTL effects can be located in small populations of 50-100 lines rather than in bigger segregating populations. (3) Interesting QTL effects can immediately be studied in detail on all omic levels by comparing a single IL which covers the QTL effect against the recurrent parent which lacks the QTL effect. (4) The ILs are immediate launching pads to embark on map-based cloning of verified QTLs.

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P1.6

MAJOR SPOT TYPE AND NET TYPE NET BLOTCH RESISTANCE GENES IN THE ETHIOPIAN BARLEY LINE CI 9819

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Net blotch of barley (*Hordeum vulgare* L.), caused by the fungal phytopathogen *Pyrenophora teres* Drechs. f. *teres* Smedeg., constitutes one of the most serious constraints to barley production world-wide. Two forms of the disease, the net form caused by *P. teres* f. *teres* and the spot form caused by *P. teres* f. *maculata*, are differentiated based on the type of symptoms on leaves. Several barley lines with major gene resistance to net blotch have been identified. Earlier we mapped one of these in the cross Rolfi x CI 9819 to barley chromosome 6H using a mixture of four Finnish isolates of *P. teres* f. *teres.* In the present study we used the same barley progeny for mapping resistance to four spot type isolates and four net type isolates of *P. teres*. With all net type isolates a major resistance gene was located on chromosome 6H in the same position as described previously, explaining up to 88% of the phenotypic variation of infection response in the progeny. We hereby designate this gene *Rpt5.* Several minor resistance genes were located on chromosomes 1H, 2H, 3H, 5H and 7H. These minor genes were not genuinely isolate specific but their effect varied among isolates and experiments. When the spot type isolates were used for infection a major isolate-specific resistance gene was located on chromosome 5H, close to microsatellite marker HVLEU, explaining up to 84 % of the phenotypic variation of infection response in the progeny. We hereby designate this gene *Rpt6.* No minor gene effects were detected to spot type isolates. The Ethiopian two-rowed barley CI 9819 thus carries at least two independent major genes for net blotch resistance: *Rpt5* active against net type isolates and *Rpt6* against specific spot type isolates. We are currently in the process of developing a larger mapping progeny for saturation mapping in the region around *Rpt5.*

P1.7

ESTIMATING GENETIC DIVERSITY IN WHEAT LANDRACES USING ISSR MARKERS

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Landraces are heterogeneous, genetically rich, dynamic populations that have evolved under nature and farmers selection. They are characterized by wide intra-population diversity that contributes to their adaptability to adverse environments. Proper description of genetic diversity of landraces is imperative for efficient exploitation in plant breeding programmes. Molecular markers have proved to be useful tools for understanding the genetic composition of agricultural crops and have become preferable methods for evaluating genetic diversity. In the present preliminary study, six ISSR (Inter-Simple Sequence Repeat) primers were applied for evaluating genetic diversity within and among Greek durum wheat landraces. Based on four morphological traits namely 'awn presence', 'awn color', 'glume hairiness' and 'glume color', landraces could be subdivided into 2 to 4 subpopulations and a total of 48 subpopulations were selected. Cluster analysis was carried out using the Unweighted Pair-Group Method with Arithmetic Means (UPGMA) based on Jaccard's similarity coefficient. The ISSR primers yielded a total of 195 bands, 182 of which were polymorphic. The majority of subpopulations derived from the same landrace could be grouped together. Conclusively, the ISSR technique is a powerful tool to describe not only the genetic diversity among durum wheat landraces but also to distinguish the closely related subpopulations.of developing a larger mapping progeny for saturation mapping in the region around *Rpt5.*

Speakers' abstracts

Session 2: WG2: Accessing the Physical Genome for Sustainability and Quality (PhysGen)

S11

TOWARDS PHYSICAL MAPPING OF THE BARLEY (HORDEUM VULGARE L.) GENOME

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The barley genome comprises over 5 billion bp – almost double the size of the human genome. Despite of this size it represents the least complex triticeae crop genome (diploid, n = 7) with a wealth of genetic and genomics resources developed throughout the past decade (e.g. >460,000 ests, > 20,000 unigenes present on an Affymetrix chip, transcript map with >1000 genes, 80,000 gene-containing bacs identified, several tilling populations).
Although feasible, map-based gene isolation in barley is tedious and time consuming. A physical map of the barley genome would accelerate the process of isolating agronomically important genes. It also would provide the required template for sequencing the barley genome - a resource which is ultimately in order to reach a profound understanding of crop plant biology.
The first step towards the construction of a genome-wide physical map of barley has been initiated as a collaborative project between IPK gatersleben (BAC DNA isolation, high information content fingerprinting hicf and fingerprint assembly), the Australian Center of Plant Functional Genomics (BAC libraries; ACPFG) and the Technical University of Munich (bioinformatics support). The aim of the project will be the high-information-content-fingerprinting of 350,000 bac from three different libraries (hindiii, ecori, and bamhi) representing up to 10x genome coverage. The project started in June 2006 based on availability of two BAC-libraries (hindiii) (yu et al., 2003; and unpublished data acpfg). Both libraries showed an average insert size of approx. 100 kb. Since June 2007 a further bac-library (partial digestion with ecori) from Clemson University Genomics Institute (CUGI) consisting of 147,000 clones with an average insert size of 125 kb became available. construction of a bamhi-library is underway (ACPFG).
We will provide a report about the most recent status of the project. Currently (July 2007), about 90,000 BAC-fingerprints have been accomplished (clones from hindiii-libraries that equal about 1.8 x genome coverage). Given a continuous progress, this number will increase to about 150,000 fingerprints by october 2007. Fingerprinting of clones from the new ecori-bac library and of 80,000 gene-containing BAC-clones has just been initiated.

S12

DEVELOPMENT AND PHYSICAL (BIN) MAPPING OF SSR MARKERS FOR THE SHORT ARM OF RYE CHROMOSOME

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Simple sequence repeats (SSRs) are valuable molecular markers in all organisms. SSRs are particularly important in self-pollinating crops like wheat because of their high degree of polymorphism compared toother molecular markers. 1RS is part of the rye and triticale genome. Since, in addition, 1RS is today present in hundreds of wheat cultivars around the world, it can even be regarded as part of the wheat germplasm. We found 100 polymorphic (rye) SSR markers with a microsatellite marker development procedure involving nebulization of 1RS specific genomic DNA and a highly efficient SSR enrichment protocol. Using 1RS deletion lines, 50 of these markers could be assigned to one of three 1RS bins.In addition 50 further markers, not polymorphic in the tested rye cultivars, have been mapped to these bins. The 1RS specific SSR markers might help to identify genes responsible for fertilization control in rye and the physical (BAC) mapping of 1RS, providing valuable anchor markers. The 50 polymorphic (rye) SSR markers amplifying both in wheat and rye might contribute to our understanding of the close relationship between wheat and rye.

S13

GENETIC, PHYSIOLOGICAL AND MOLECULAR ANALYSES OF WHEAT LINES CARRYING FLOWERING TIME GENES ON CHROMOSOME 3B

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The presence of a gene/genes delaying flowering time and possibly enhancing winter survival was originally detected in chromosome 3B of a Czech alternative wheat variety e ská P esívka (CP 3B) by substitution line analysis in the background of a spring variety Zlatka. A more detailed analysis of the influence of the chromosome 3B substitutions has led to the hypothesis of the presence of an eps gene/s that could be homoeologous to a previously known eps gene on chromosome 3A. To describe the phenotype more precisely and to define the point where the flowering time gene acts, SSR validated lines of two spring wheat substitutions - Zlatka (CP3B), Sandra (CP3B) - and two winter wheat substitutions - Vala (CP3B), Zdar (CP3B) were grown under different photoperiod and vernalization regimes. Half of the materials were planted into the field with natural day length following 8-weeks of full vernalization or zero vernalization, while the other half consisted of fully vernalized plants grown in a covered field plot under a 10-hours´photoperiod. Significant differences in heading time, up to 19 days, occurred between parental varieties and the substitution lines under short day conditions which confirmed the presence of a gene sensitive to photoperiod in all the lines tested. Vernalization influenced the time to heading and lowered the differences in heading, which, though, remained significant under long days in the winter wheat materials. The earlier start of the individual stages of spike development, on average by 5 to 7 days, was detected in Zlatka compared to Zlatka (CP 3B) grown under short days, and it took place during the period of reproductive development of the plants. This effect was confirmed in a repeated experiment in the following season. Tests of frost resistance under natural winter conditions revealed enhanced survival of the substitution line Vala (CP 3B) compared to the original variety Vala, while the other tested substitution lines (Zlatka (CP 3B), Sandra (CP 3B) and Zdar (CP 3B) were unchanged in sensitivity to frost. Repetition of the test in a combined natural/ laboratory conditions revealed the same effect. SSR molecular fingerprinting of the Zlatka (Zlatka 3B/CP 3B) single chromosome recombinant lines was carried out to develop a comprehensive map of chromosome 3B, and QTL analysis was done for flowering time on the F3 and F4 generations of the lines). A QTL was mapped in both generations into a position near the centromere of 3B. The genetic map of 3B has been compared with a previous map developed using the Sandra (Sandra 3B/CP 3B) single chromosome recombinant lines, and other 3B maps available at the JIC based on the recombinant doubled haploid populations of UK winter wheats Spark x Rialto and Charger x Badger.

S14

IDENTIFICATION OF CDNAS INVOLVED IN WINTER WHEAT COLD ACCLIMATION PROCESS

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To identify novel genes involved in cold acclimation, a cost effective ECL-based macroarray platform was developed and utilized for screening a cDNA library from cold acclimated winter wheat crown tissue. Randomly selected 920 cDNA clones were PCR amplified, spotted on nylon membranes and hybridized with ECL labeled 14 days cold acclimated and non acclimated wheat crown cDNAs. Data analysis identified 89 clones whose mRNA levels were elevated by more than two fold in the cold acclimated crown tissue. The expression pattern of selected differentially regulated clones was confirmed by northern blot. ESTs from the up-regulated clones were collected and compared against the public sequence databases. In addition to proteins with well characterized functions in the cold acclimation process, cDNA clones that showed homology to proteins that have not previously been associated with cold acclimation were identified. An example of functional analysis in yeast and transgenic Arabidopsis of a cold acclimation induced cDNAs encoding a novel GSK3/shaggy-like kinase will be presented.

S15

UNRAVELING THE GENETIC BASIS OF SEVERAL QUALITATIVE AND QUANTITATIVE AGRONOMIC TRAITS DERIVED FROM WILD EMMER WHEAT

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Wild emmer wheat, *Triticum dicoccoides* (genome AABB), the progenitor of cultivated wheat, offers a rich source of allelic diversity in many agronomic traits valuable for improvement of cultivated wheat. The main objective of the studies conducted by our group is to unravel the genetic basis of potentially important qualitative and quantitative traits (e.g. disease resistance, drought resistance, grain protein and grain mineral content) derived from wild emmer wheat. Traditional approaches for exploitation of wild genetic resources are usually very slow. The advanced genomic technologies available today can help to accelerate the utilization of wild germplasm for crop improvement. We have conducted genetic mapping studies of novel stripe rust and powdery mildew resistance genes derived from wild wheat. The availability of wheat genomic resources (e.g. genetic maps, ESTs, BAC libraries, etc.) enabled us to initiate positional cloning efforts targeting the stripe rust resistance gene, Yr15, and a novel powdery mildew resistance gene, temporarily designated PmG3M, both derived from wild emmer wheat. We are currently developing high resolution maps of these genes using the wheat-rice colinearity approach. Furthermore, QTL mapping have been used to dissect drought resistance and high grain protein content in wild emmer x durum wheat populations. Physical mapping and positional cloning of Gpc-B1, a QTL for high grain protein content, were conducted in collaboration with J. Dubcovsky at UC Davis. The establishment of wheat-rice colinearity in the Gpc-B1 region enabled us to develop a complete physical map of this gene region and to accelerate the positional cloning of the target gene. The Gpc-B1 allele of *T. dicoccoides* was found to confer pleiotropic effects that included earlier senescence and increased grain mineral concentrations (e.g., Zn and Fe). The functional Gpc-B1 allele derived from wild emmer wheat is absent in cultivated durum and bread wheat germplasm, indicating a high potential for crop improvement. These studies demonstrate the potential of wild emmer wheat gene pool for improvement of cultivated tetraploid and hexaploid wheats, as well as the contribution of the recently developed genomic tools for the utilization of wild wheat germplasm.

S16

IDENTIFICATION AND CHARACTERIZATION OF CONSERVED ORTHOLOGOUS MARKERS BETWEEN BARLEY AND BRACHYPODIUM

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Brachypodium distachyon has been proposed as a model system for functional genomics in temperate grasses because of its biological features and compact genome size. Molecular phylogenetic studies have suggested that the genus *Brachypodium* is closely related to *Triticeae*, which include barley and wheat. This new model plant has been employed to build a new molecular marker map, with the aim to link it to existing barley maps. A physical map of barley is being developed in European institutions, and the availability of informations of colinearity between cultivated barley and *Brachypodium* could be useful for accelerating the cloning of barley QTLs. With this aim in mind, an F2 population of *B. distachyon* derived from a cross between the line Bd1-1 and the line Bd3-1 has been used to build up a framework map mainly based on AFLP markers. As a second step, a research of EST-derived, and conserved orthologous markers between barley and *Brachypodium* has begun, in order to anchor the new map with the barley Nure x Tremois and Vada x L94 maps.

S17

PRODUCTION OF SUBGENOMIC BAC LIBRARIES AND OTHER RECENT ACTIVITIES RELATED TO CHROMOSOME SORTING IN CEREALS

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Chromosome sorting using flow cytometry was developed in barley, polyploid wheat and rye to simplify genome analysis and gene cloning. Any chromosome that can be discriminated from the remaining ones based on DNA content can be sorted. Thus, only chromosome 3B can be sorted from polyploid wheat, and only chromosomes 1H and 1R can be sorted from barley and rye, respectively. However, the use of telosomic lines facilitates sorting chromosome arms of the remaining chromosomes in polyploid wheat; the only exception being the long arm of chromosome 5B, which can only be sorted as an isochromosome. Wheat-barley telosome addition lines facilitate sorting arms of the remaining barley chromosomes (2H - 7H); the use of wheat-rye chromosome addition lines makes it possible to sort the remaining chromosomes of rye (2R - 7R). The ability to isolate particular chromosomes and chromosome arms offers attractive applications for targeted genome analysis. Flow-sorted chromosomes are suitable for high-resolution cytogenetic mapping using FISH. Other applications include physical mapping using PCR and microarrays, and targeted isolation of molecular markers. The most attractive application has been the production of chromosome- and chromosome arm-specific BAC libraries. Compared to their genomic counterparts, the libraries comprise smaller number of clones and hence are easier to maintain and use. Moreover, their subgenomic nature simplifies assembly of physical contig maps and in polyploid wheat avoids problems caused by the presence of homoeologous genomes. Although the first subgenomic BAC library was published only three years ago, the results obtained so far clearly demonstrate the potential of chromosome-based genomics. The subgenomic BAC libraries have been used to develop markers from specific chromosomes, to construct physical maps, for positional gene cloning and in comparative analysis of wheat genomes. In addition to simplifying the genome sequencing efforts, the chromosome-based approach enables division of labour. With the exception of a BAC library from the short arm of rye chromosome 1R (1RS), all existing subgenomic BAC libraries were made from hexaploid wheat. These include a library from a group of D-genome chromosomes (1D, 4D and 6D), two libraries from chromosome 3B, and libraries from short arms of chromosomes 1B (1BS), 3A (3AS) and 3D (3DS). With the insert sizes ranging from 80 to 100kb the libraries provide excellent coverage of specific genome regions. Recent results with 3DS BAC library with the average insert size over 110kb indicate that it is possible to improve the insert size. A major challenge is to produce BAC libraries from all chromosome arms of wheat. This work has been supported by the Czech Science Foundation (awards no. 521/05/0257, 521/05/P257, 521/05/H013, 521/06/P412, 521/06/1723, and 521/07/1573) and Ministry of Education Youth and Sports of the Czech Republic (awards ME884 and LC06004).

S18

TOWARDS FINE MAPPING OF RESISTANCE GENES TO SEPTORIA TRITICI LEAF BLOTCH IN HEXAPLOID AND DIPLOID WHEAT

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One of the key wheat diseases under investigation at RRes and JIC is *Septoria tritici* leaf blotch (STB) disease caused by *Mycosphaerella graminicola*. At least thirteen genes (Stb1 to Stb12, and Stb15) specifying race-specific resistance to *M. graminicola* in hexaploid wheat have been mapped but none has been isolated. One of these genes, Stb6, is present in many wheat genotypes used as sources for STB resistance and therefore it is likely contributing to resistance in the field by lowering STB disease levels. The Stb6 is also particularly interesting from the fundamental point of view as it functions in the absence of hypersensitive cell death (HR) and rather appears to confer resistance by avoiding or preventing this type of host programmed cell death (PCD). PCD, as we have recently demonstrated, is induced by *M. graminicola* in both, hexaploid and diploid einkorn wheat (*Triticum monococcum*), and is required for successful fungal sporulation during a compatible interaction (Keon et al., 2007). More broadly, the resistance proteins that function against necrotrophic and/or hemibiotrophic plant pathogens remain elusive particularly when compared to the situation for those that function against biotrophs. Our recent findings raise the possibility that other Stb genes may operate in the same fashion, and isolation of Stb6 will provide an opportunity to test this hypothesis. An update on the recently initiated project to fine map and clone Stb6 will be presented. We will also present data on molecular genetic characterisation of race non-specific resistance to *M. graminicola* that we have recently shown to exist in *T. monococcum*.

References:

Keon J, Antoniw J, Carzaniga R, Deller S, Ward JL, Baker JM, Beale MH, Hammond-Kosack K, Rudd JJ (2007) Transcriptional adaptation of *Mycosphaerella graminicola* to programmed cell death (PCD) of its susceptible wheat host. Mol. Plant Microbe Interact. 20, 178-193.

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S19

HOW TO PRESERVE, MAINTAIN AND EXPLOIT PLANT GENOMIC RESOURCES GENERATED BY INTERNATIONAL RESEARCH

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During the last decade various plant genome projects, studying models as well as crops species, have been achieved. These projects have resulted in the production of numerous genomic collections (BAC, cDNA...) and related data (whole genome sequence, EST, BAC end sequences...). The French Plant Genomic Resource Centre (CNRGV) created in France in 2004 by the INRA (French National Institute for Agricultural Research), is not only a repository centre for all these plant genomic collections but also a service provider dedicated to the scientific community. These genomic collections are the starting point for functional annotation of genome (i.e. the formulation and testing of hypothesis that ascribe functions to genomic sequences). The construction of BAC libraries has allowed the cloning of several genes through map-based cloning. However, positional cloning remains slow and tedious since genomic resources are rarely adapted for efficient, high throughput and specific screening. Screening of libraries is mainly made by hybridization on high density filters which suppose to work on large number of membranes. The CNRGV is developping rapid, efficient and cheap tools to exploit BAC libraries in order to isolate positive clones located in regions of interest. Three-dimensional pools for wheat and tomato BAC libraries are under process. PCR screening of these pools could be used for isolation of genes of interest, construction of physical map of a region, comparison of genomic studies between crop and model species. The objective of the CNRGV is to produce efficient tools for genome analysis, physical mapping, map-based cloning and sequencing projects and made them available for the scientific community. More information about the CNRGV, available collections and services can be found at http://toulouse.inra.fr/cnrgv/.

Poster abstracts

Session 2: WG2: Accessing the Physical Genome for Sustainability and Quality (PhysGen)

P2.1

THE INTERNATIONAL BARLEY SEQUENCING CONSORTIUM (IBSC) - PROGRESS TOWARDS EFFICIENT GENE ISOLATION AND GENOMIC SEQUENCING IN BARLEY

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Barley ranks number five in world crop production. Its grain is predominantly used for animal feed and for the production of malt, which forms the raw material for the brewing and distilling industries and it is suited to become an integral part of a bio-based economy. Cultivation of the cool season crop barley reaches higher latitudes, higher altitudes and deeper into deserts than of any other major crop species. Thus, it represents a central part in the vast majority of agro-ecosystems in temperate regions. Due to its salient agricultural importance, its ample genetic variation and its multiple uses in the food and feed industry, barley has attracted the attention of many geneticists throughout the world. It has been used as a genetic model organism, since its seven chromosomes represent the base complement of species within the Triticeae subfamily including wheat and rye. The large genome of barley consisting of over 80% repetitive DNA has long hampered efficient progress in barley genomics. With the goal to change this situation, the International Barley Genome Sequencing Consortium (IBSC) was formed in year 2006 aiming to reach for a coordinated fund raising at the national and international level and for a consensus research plan approaching barley genome sequencing. An overview of the IBSC research agenda and a status report about the recent achievements in barley genomics will be reviewed.

P2.2

UNDERSTANDING THE PARTIAL RESISTANCE CONFERRED BY THE PCH1 GENE TO OCULIMACULA YALLUNDAE IN WHEAT : MICROSCOPIC AND MOLECULAR APPROACHES

Blein M.[1], Paillard S.[1], Levrel A.[1], Lemoine J.[1], Wei L.[1], Jahier J.[1], Chalhoub B.[2], Coëdel S.[2], Muranty H.[1], Barloy D.[1]

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Cereal crops in many parts of the world are often infected by fungi which attack the base of plants. One of the most important of these stem base diseases in wheat is eyespot, caused by two closely-related species of Oculimacula. These pathogens can cause severe damages on wheat crops, affecting both yield and quality of grains. Because Oculimacula sp. can easily adapt to fungicides, genetic resistance appears to be the most efficient way to control eyespot disease. An improved understanding of genetics and mechanisms of resistance to Oculimacula sp. is essential to devise more sustainable approaches to eyespot control. This project focuses on the Pch1 gene, which is a major gene conferring partial resistance to O. yallundae. This gene, introduced from Aegilops ventricosa into wheat, is the most efficient resistance gene and the most widely used by wheat breeders. The objectives of this project are to identify the defence mechanisms involved in a partial resistance conferred by the Pch1 gene and to isolate the Pch1 gene. First, we characterized the interaction between a wheat line carrying the Pch1 gene and O. yallundae. We established a relationship between symptom development and the changes occurring in pathogen development and plant responses during the interaction between a partially resistant wheat line carrying Pch1 and O. yallundae. To do this, we studied simultaneously the timing of symptom development, pathogen development and histological and molecular plant responses in eyespot inoculated and mock-inoculated partially resistant wheat. During the asymptomatic phase corresponding to coleoptile colonisation, local H2O2 generation and callose deposition were observed and PR genes were up-regulated. During the symptomatic phase beginning with the first leaf sheath attack, natural browning occurred in epidermal cells and the PR genes were more strongly up-regulated. These results show that changes in the pathogen development and plant responses occurred during the transition period between the asymptomatic to the symptomatic phases. Second, we initiated the map-based cloning of Pch1 in a population of 135 recombinant-inbred lines derived from a cross between Ae. ventricosa 10 (partially resistant, Pch1) x Ae. ventricosa 7 (moderately susceptible). The Pch1 gene is located at the distal part of the long arm of chromosome 7D. Using Bulk Segregant Analysis, a total of 28 AFLP markers were identified close to the Pch1 gene. Our present work focuses on the development of codominant PCR-based markers for the fine mapping of Pch1 in a large Ae. ventricosa population. This includes the development of STS (sequence tagged site) markers from AFLP markers and the use of the synteny between Poaceae and rice to derive markers from wheat EST sequences or directly from rice gene sequences. Future work will focus on the identification of defence mechanisms

involved in the partial resistance controlled by the Pch1 gene during the asymptomatic and symptomatic phases. To carry out this study, we developed isogenic lines for the Pch1 gene and we will use a combination of 'omics' approaches (transcriptomic arrays, metabolomics and proteomics).

P2.3

BULK SEGREGANT (BSA) BASED TRANSCRIPTIONAL PROFILING OF SOIL-BORNE CEREAL MOSAIC VIRUS RESISTANCE IN HEXAPLOID WHEAT (*TRITICUM VULGARE SSP. AESTIVUM*)

Perovic D.[1], Winter A.[2], Weyen J.[2], Förster J.[2], Devaux P.[3], Hariri D.[4], Guilleroux M.[4], Scholz U.[5], Graner A.[5], Ordon F.[1]

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Bulk segregant (BSA) based transcriptional profiling of Soil-borne cereal mosaic virus resistance in hexaploid wheat (Triticum vulgare ssp. aestivum) Perovic D1, Winter A5, Weyen J2, Förster J2, Devaux P3, Hariri D4, Guilleroux M4, Scholz U5, Graner A5 and Ordon F1 1 Institute of Epidemiology and Resistance Resources, Federal Centre for Breeding Research on Cultivated Plants, Theodor-Roemer-Weg 4, 06449 Aschersleben, Germany 2 Saaten-Union Resistenzlabor GmbH, Hovedisser Str. 92, 33818 Leopoldshöhe, Germany 3 Florimond Desprez, 3, rue Florimond Desprez, 59242 Cappelle en Pévèle, France 4 INRA, RD 10, Versailles Cedex, France 5 Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany Soil-borne cereal mosaic virus (SBCMV) belonging to the Furoviruses is a serious constraint to winter wheat cultivation in Europe. Due to transmission by the plasmodiophorid Polymyxa graminis, which has been detected down to a soil depth of 60 cm, chemical measures are neither effective nor acceptable for economical and ecological reasons. Therefore, the only possibility of controlling this virus is breeding and growing of resistant cultivars. In order to get detailed information on the genetics of resistance, which is a translocation resistance, a set of different DH-populations has been analysed and it turned out that resistance to SBCMV is inherited in a monogenic manner. Using bulked segregant analysis, resistance derived from cvs. 'Tremie` and `Claire` was mapped on chromosome 5D. In addition to mapping, transcription profiling was carried out in parallel by macro-array analysis and cDNA-AFLP. Hybridisation of a barley cDNA array comprising 10.000 unigenes with wheat RNA isolated after SBCMV infection revealed 80 genes differentially expressed in roots, 11 in hypocotyls and four in leaves. By the use of median and quantile normalization methods, seven genes in roots and four in hypocotyls and leaves, respectively, were identified as differentially regulated in resistant and susceptible bulks. By cDNA AFLP 8 differentially expressed fragments were detected. Out of 16 genes differentially expressed between the resistant and susceptible bulk, 6 revealed the best homologues at syntenic rice chromosome 3. All differentially expressed genes will be screened for polymorphism and subsequently genetically mapped. The work was supported by a grant in the Community´s Sixth Framework Programe (WHEATPROTECT, EU contract number COOP-CT-2004-512703). These results and this publication reflect only the author's view and the Community is not liable for any use that may be made of the information contained in this poster.

Speakers' abstracts

Session 3: WG 2A Bionformatics for WG1 and WG2

S20

FUNCTIONAL SIGNIFICANCE OF THE STRUCTURAL REARRANGEMENTS OF CANDIDATE GENES

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In the past few years the role of introns in regulation of gene expression became more evident. Variable and conserved intronic sequences harbour several functionally important sites including matrix attachment regions, transcription factor binding sites (TFBSs), chromosome-level regulatory regions, DNase I hypersensitive sites, enhancers and repressors. Intronic regulatory elements can also contain insulators that influence expression of neighbouring genes and genes transcribed in anti-sense direction. Strategy of our laboratory aimed on evaluation of eventual functional significance of the structural rearrangements of non-coding regions in the candidate genes will be the topic of the report. We focus our attention on the pattern of transcription factor binding sites (TFBSs), modeling of the DNA and RNA secondary structures, prediction and proof of DNA curvatures, mapping of interaction sites with tightly bound proteins. The approach will be explained on example of barley Bmy 1 gene intron III. Inter- and intra-haplotype variability of the whole intron and microsatellite portion and functional consequences of sequence variations in this region will be discussed.

S21

HANDLE THE JUNK - A UNIFIED CLASSIFICATION SYSTEM FOR EUKARYOTIC TES

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Our knowledge of the structure and composition of genomes is rapidly progressing in pace with their sequencing. The emerging data show that a significant portion of eukaryotic genomes is composed of transposable elements (TEs). TEs, because of their multiple means of replication, mutability and abundance, are extremely diverse. This diversity, combined with an earlier lack of regularity in nomenclature presents a confusing landscape. Given the abundance and diversity of TEs, the speed at which large quantities of sequence data are emerging presents an enormous identification and annotation problem. No unified system has been proposed, such as exists for the genes (e.g., Gene Ontology), which can be easily applied to the millions of TEs that will have to be annotated. Here, we propose a hierarchical system that can be easily applied by non-experts. The classifications are based on the transposition mechanism, as well as on sequence similarities and structural relationships. A key component of our system is a naming convention: a three-letter code with each letter respectively denoting Class, Subclass, and Superfamily; the family (or subfamily) name; the sequence (database accession) on which the element was found; the "running number" of that element on the sequence. The system and nomenclature will be kept up-to-date at www.wikiposon.org.

S22

SEQUENCING A LARGE GENOMIC REGION IN WHEAT

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In an attempt to produce one of the largest genomic sequences from wheat so far, a contig of 20 BAC clones from wheat chromosome 3BS was targeted for complete sequencing. The BAC clones were sequenced individually by shotgun sequencing to an approximately 6-fold coverage. Here, we describe the problems encountered and the methods developed for the finishing phase of this sequencing project.
Due to the highly repetitive nature of the BAC clones, the initial assembly yielded approximately 30 sequence contigs per BAC clone. Sequences from overlapping regions of BACs were combined which allowed to close approximately half of the gaps in the overlaps. The major cause for gaps turned out to be sequences that are problematic for sequencing. For example, almost all LTRs of Angela and WIS retrotransposons contain a region of about 400 bp that was not covered by the sequences or where sequence quality was very low. Similar problematic regions were found in tandem repeat arrays inside CACTA transposons.
Sequence contigs could be arranged in their correct linear order into supercontigs using a set of strategies. These included information from forward and reverse reads of shotgun clones and from detailed repeat annotation. There, we used information from target site duplications of transposable element as well as from their nesting patterns. The presented strategies allowed to arrange more than 90% of the sequence contigs in the BACs processed. The produced working models were used for primer design for targeted closing of the gaps.

Poster abstracts

Session 3: WG 2A Bionformatics for WG1 and WG2

P3.1

TAKING UP THE CHALLENGE OF LARGE GRASS GENOMES: THE MIPS ANNOTATION INFRASTRUCTURE FOR COMPARATIVE ANALYSES

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The grass genome derived sequence flood will pose manifold bioinformatic challenges in terms of assembly, automated and validated genetic element annotation pipelines, computing time, cross species data integration and visualization. We have established gene and repeat detection pipelines, which provide together with our generic database system plantsDB (http://mips.gsf.de/projects/plants) a solid infra-structure and expertise to meet these challenges. The MIPS high throughput gene annotation pipeline combines several complementary gene detection programs such as Genmark, Fgenesh++/Protmap, Glimmer HMM and GenomeThreader. The individual gene prediction programs are trained and validated for each species using EST confirmed high quality gene sets. All genes are marked with a quality flag reflecting the underlying evidence for the respective gene call. Functional annotation is undertaken by homology based information transfer as well as protein domain analyses. Syntenic regions between different species or segmental duplications are identified by established high throughput pipelines. Clear-cut syntenic relationships help to distinguish orthologous from paralogous genes and are an important data resource for a wide range of comparative studies. The main components of large plant genomes are repetitive elements, covering 50 to 90 percent of the sequence. Nowadays there are not only seen as mere junk, but as essential players in genome evolution. A comprehensive repeat annotation provides a valuable data source to unravel such evolutionary processes. Our repeat annotation concept is based on mips-REcat a generic repeat element classification catalog and mips-REdat, an exhaustive database of plant repeat elements. The generic pipeline ANGELA combines intrinsic repeat detection approaches with homology based methods followed by the identification of nested structures and the timing of insertion events. An appropriate visualization of complex data can uncover hitherto unknown patterns and relationships, especially for segmental duplications and syntenic regions. On the chromosome level heat maps are used, to depict the distribution of selected features, like element densities or insertion ages. We use the Apollo synteny viewer with own tiers and customized color codes to gain a more thorough insight into the spatial interplay between different types of genetic elements. Taking established resources and upcoming data together we are on the way towards system level comparative plant genomics.

Speakers' abstracts

Session 4: WG3: Implementation of Genomics Approaches for Understanding Cereal Traits

S23

GENES REGULATING SEED DEVELOPMENT

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S24

CANDIDATE GENE AND FUNCTIONAL GENOMICS APPROACHES FOR THE MOLECULAR DISSECTION OF BARLEY DEVELOPMENT

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Developed as a collaboration between the University of Milan and the Parco Tecnologico Padano (PTP), research in our group focuses on the genetic and molecular dissection of barley development as a mean to identify useful genes for the manipulation of plant architecture. Target traits include tillering, spike morphology and development of leaves and floral bracts. In order to identify and characterise genes involved in these processes we have been exploiting a wide collection of developmental mutants, combining candidate gene (CG) and functional genomics approaches. Identification of candidate genes has been based on two complementary strategies: 1. SYNTENY-BASED STRATEGY. Forty developmental mutant loci were positioned onto a molecular map of the barley genome. Based on map position, rice syntenous regions were defined and CGs identified for 24 barley mutants. Using this approach, the barley ortholog of the rice FRIZZY PANICLE (FZP) gene was isolated as a candidate for the branched1 (brc1) mutant altered in inflorescence architecture. Allelic comparisons and co-segregation analysis on the progeny of a wild-type x brc1 cross support the correspondence between barley FZP and the mutant locus. 2. MOLECULAR APPROACHES. CGs for meristem function and lateral organ development have been identified through molecular approaches, using as a starting point the Hooded (K) mutant. The K phenotype is due to the duplication of a 305 bp enhancer element in intron IV of the Barley knox3 (BKn3) gene. Knox (knotted1-homeobox) genes are normally expressed in shoot meristems and downregulated in lateral organ primordia. In K barley, the 305bp enhancer element causes ectopic expression of Bkn3 in the lemma-awn transition zone leading to the formation of an ectopic meristem that develops into an epiphyillic flower. This provides an ideal experimental system to investigate mechanisms of knox gene regulation. A one hybrid screen aimed at isolating putative regulators of the BKn3 gene uncovered 4 proteins capable of interacting with the 305 bp element (K Intron Binding Proteins, KIBPs). Molecular and functional analyses of these genes suggest they may mediate the cross-talk between the knox network and hormonal pathways. Insight into KIBP gene function could be gained by analysis of mutant phenotypes, but to date no clear association between these genes and existing barley mutants could be established. For the functional validation of CGs, we are exploiting a TILLING functional genomics resource developed for barley cultivar Morex by the group of DISTA (University of Bologna) in collaboration with the PTP Genomics Platform. By these means, mutations at selected CGs can be identified and are currently being characterised.

S25

CHANGES IN THE PROTEOME PATTERN DURING GRAIN DEVELOPMENT OF CEREALS

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A study of changes in barley proteome during grain-filling performed by 2D electrophoresis is presented with the use of Pixel based analysis of Multiple images for identification of Changes (PMC) followed by manual recognition of spots for a material with large batch to batch migration differences. A par of near isogenic barley lines, Betzes and Wanubet, were grown under controlled conditions and grains were harvested from 10 to 45 days after anthesis with 5 days interval. 2D electrophoresis of Tris-soluble proteins and chemical analysis were performed. The PMC approach for analysing 2D images was performed within batches and involved aligning, normalisation, unfolding to 1D pixel vectors, analysing pixel vectors by multivariate data modelling, and refolding back to the image domain for visualization and interpretation. From the refolded images the proteins changing over time were localized. From this, selected spots are subjected to further identification by MALDI-TOF.

S26

FUNCTIONAL PROTEOMICS OF BARLEY SEEDS: INTEGRATION OF THE PROTEOME WITH THE GENETIC MAP, GENE EXPRESSION AND ENZYME ACTIVITIES

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Two-dimensional gel electrophoresis was used to screen a set of spring barley cultivars for differences in seed protein profiles. Cultivars were characterised with respect to 72 microsattellite (SSR) markers. More than 60 protein spots varied among the cultivars. Forty-eight spots segregated in doubled haploid lines derived from two of the cultivars, enabling mapping of 12 linkage groups to chromosomes, directly coupling the proteomic and genetic maps. Mass spectrometry identified proteins in over 40 of the varying spots and the molecular basis for spot variations was determined in several cases to be amino acid changes resulting from SNPs. Since these coding SNPs can alter the properties of the affected protein, they represent a direct method for correlating cultivar properties with the genome and demonstrate that proteomic data contains valuable genetic information.
To identify proteins involved in germination, dissected embryo and aleurone layers were analysed. In embryo, many germination-related changes involved enzymes with roles in oxidative stress. The appearance of an ascorbate peroxidase spot was in agreement with assay of increasing enzyme activity in the embryo protein extracts and gene expression data. A study of the thioredoxin system in germinating seed tissues led to cloning, expression and characterisation of two isoforms each of thioredoxin h and thioredoxin reductase. The aleurone layer responds to gibberellic acid (GA) produced by the embryo by synthesizing hydrolytic enzymes that are released to the endosperm. Aleurone layers can be separated from the other seed tissues and maintained in culture. In this system, proteins released from the aleurone layer accumulate in the culture medium. Changes in response to GA were analysed using one and two-dimensional gel electrophoresis, western blotting for specific proteins and mass spectrometry. Proteins in soluble and plasma membrane-enriched fractions were identified. In the first proteome analysis of plasma membranes from a seed tissue, over 40 proteins associated with barley aleurone plasma membranes were identified, some with more than 10 transmembrane domains and some with unknown functions.

S27

ANALYSIS OF ENZYME ACTIVITIES, WORT SUGARS AND BARLEY MALTING QUALITY

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We describe the use of a composite spring barley population, formed from the amalgamation of several small doubled haploid populations from pairwise crosses between elite genotypes, to analyse the genetics of a range of malting quality traits and identify QTLs associated with their genetic control. The population was grown at two locations over three years in field trials and grain samples from each plot were micro-malted. The micro-malts were analysed for parameters such as hot water extract, total and soluble nitrogen, fermentability and diastatic power. Wort samples from each micro-malt were analysed for the amounts of the sugars fructose, glucose, maltose and sucrose and the activities of several key malt enzymes, such as beta-glucanase, beta amylase and limit dextrinase.
Characters such as fermentability were found to be highly dependent upon wort sugars, principally maltose. The activity levels of enzymes in the malting process that are regulated by GA were also found to be closely related.
The composite population was also genotyped with a range of SSR and S-SAP markers, which were then used to identify associations with the phenotypes that we had studied. Significant associations were found for almost all of the characters measured with several markers being associated with multiple phenotypes, confirming the inter-dependence of some of the characters.

S28

FROM QTLS TO GENES: FUNCTIONAL GENOMICS OF FREEZING TOLERANCE IN CEREALS

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There is a long-term interest to identify the genes behind QTLs affecting abiotic stress tolerance. A genetic map of major loci and QTLs affecting stress tolerance in *Triticeae* identified the crucial role of the group 5 chromosomes, where the highest concentration of QTLs and major loci controlling the plant adaptation to environment (heading date, frost, drought and salt tolerance) has been found. Extensive molecular biological studies have led to the cloning of many stress-related genes and the expression of some of them was shown to be linked to stress-tolerant QTLs, suggesting that these genes may represent the molecular bases of stress tolerance. In wheat and barley QTLs for frost tolerance Fr-A1 and Fr-A2 were determined on chromosome 5A and 5H of wheat and barley, respectively, in orthologous positions. In case of einkorn (*T. monococcum*) on the long arm of chromosome 5Am the Fr-A2 was also identified as the main frost tolerance QTL. In each case Cbf genes were mapped at the maximum of the QTL Fr-A2. A cluster of 11 Cbf genes was mapped in a 0.8 cM region of the QTL Fr-A2 in einkorn. These genes were localised within five different BACs and were isolated. These BACs were mapped using high-density map and recombination events were found between most BACs. We report here the results of frost testing of lines carrying these recombination events, which make possible the identification of the Cbf genes responsible for frost tolerance at the Fr-Am2 locus. The most possible candidate genes for frost tolerance are: Cbf12, Cbf14 and Cbf15. Based on the sequence data gene specific primers were designed. The expression was studied in bread wheat by real-time RT-PCR. Three Cbf sequences (Cbf14, Cbf15 and Cbf16) showed significantly higher relative transcript level in single chromosome 5A recombinant lines differing for the Fr-A2 region. We recently have started a project to directly prove the involvement of the Cbf genes mentioned above in frost tolerance by transformation of wheat and rice plants. This work was supported by the Hungarian Wheat Spike Consortia (NKFP 406404) and by OTKA T046573.

S29

DISSECTION OF THE QUANTITATIVE BASIS OF YIELD, YIELD COMPONENTS AND PHYSIOLOGICAL TRAITS RELEVANT FOR ADAPTATION TO MEDITERRANEAN ENVIRONMENTS IN DURUM WHEAT

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The genetic capacity of winter cereals like durum wheat to sustain yield under highly variable rainfall patterns across locations and years is at the basis of adaptation to Mediterranean environments. Resistance to drought stress, especially in terms of yield stability under the various stress conditions that frequently occur in the drought-prone areas of the Mediterranean basin, is a main objective for durum wheat improvement. Durum wheat Italian cv. Svevo was crossed with the desert durum cv. Kofa to produce a recombinant inbred population of 249 lines (RILs). The RILs were evaluated for yield, yield components, other agronomic traits and characterized with physiological measurements in a two-year study comprising 16 trials in the following Mediterranean Countries: Italy, Spain, Morocco, Tunisia, Syria and Lebanon, under a range of water availability and yield potential (average yield of field trials ranging from 0.5 ton/ha to 5.8 ton/ha). A genetic linkage map of 254 SSRs was assembled, (2347 cM in total), and composite interval mapping for a QTL survey. Two main and highly stable chromosome regions affecting yield and related physiological traits (QTL clusters) were identified on chromosome 2BL and 3BS (both in the distal region) with the positive alleles contributed by Svevo (2BL) and by the USA line (3BS), respectively. In both cases, coincidence between the QTLs for yield and those for plant height, peduncle length, SPAD, NDVI index and thousand kernel weight were observed. The QTL cluster in the 2BL and in the 3BS chromosome arms were significantly identified in eight and seven environments, respectively. R^2 values for yield, as determined on the mean basis across the 16 environments, resulted equal to 21.5 and 13.8%, for the 2BL and 3BS, respectively.
The two QTLs could be detected in rainfed environments with as low as 1.7 and1.5 ton/ha yield capability (locations in Morocco-2004 and Tunisia-2005, respectively).

Major QTLs for heading date identified on chr. 2A, 2B (QTLs co-locating with the photoperiod response genes) and 7BS and the major QTL for plant height (chr. 1BS) had limited or null effects on yield and yield components. The results of the trials and the

corresponding QTL effects and positions will be reported in details and the QTL-environment interaction discussed.

S30

PARALLEL PIGMENT AND TRANSCRIPTOMIC ANALYSIS OF BARLEY ALBINA AND XANTHA MUTANTS

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We investigated several albina and xantha barley mutants characterized by a block in sequential steps of the chloroplast biogenesis and the corresponding wild type (WT) using the Affymetrix Barley1 GeneChip® to assess the variations of gene expression associated with chloroplast development. Chloroplast development is intimately interconnected with carotenoid and chlorophyll biosynthesis, therefore the amount of the main intermediates of the chlorophyll biosynthesis were determined in mutants and WT leaves with or without feeding with g-amino-levulinic acid (ALA). The availability of a genome wide gene expression data set and of a detailed analysis of pigment contents from the same samples has allowed us to make a parallel comparison of transcriptomic and metabolomic data. The alb-e16 mutant was characterized by a strong down-regulation of the gene coding for one of the three subunits of Mg-chelatase and by the accumulation of Proto-chlorophyllide in ALA fed leaves. Both metabolic and gene expression data are consistent with a block of the chlorophyll biosynthetic pathway before Mg-proto-chlorophyllide biosynthesis. The alb-e16 also showed a down-regulation of the gun4 homologous barley gene coding for the ChlH/Mg-ProtoIX-binding protein. The key features of the alb-f17 mutant were the down-regulation of the PorA gene encoding one of the two subunits of the POR enzyme in presence of a normal or an up-regulated expression of Mg-chelatase genes and an over-accumulation of Mg-proto-chlorophyllide in ALA fed plants. Metabolic and gene expression data are consistent with a block of the chlorophyll biosynthetic pathway before Chlide biosynthesis. The down-regulation of PorA was also associated with an up-regulation of gene coding for OEP16, a component of the POR-A Pchlide-dependent translocon complex. An additional feature of alb-f17 was represented by the over-expression of several genes involved in the phytochrome and in the phytochrome-dependent pathways. The expression analysis of the genes coding for the enzymes of the carotenoid biosynthetic pathway showed an up-regulation of Vde (violaxanthin de-epoxidase) and the down-regulation of Zep1 (zeaxanthin epoxidase) associated with the high proportion of zeaxanthin detected in all mutants suggesting that the albina and xantha mutants in presence of light are subjected to a high photo-oxidative stress. Lesions in enzymes of the tetrapyrrole biosynthetic pathway known for their involvement in plastid-to nucleus signaling lead to loss of plastid control over nuclear gene expression. The expression profile of gun3, gun4 and gun5 showed a significant variations of their expression in at least one genotype and they were used to search for other genes co-expressed across all samples. These analysis provide additional evidences on a chloroplast-dependent covariation of

large sets of nuclear genes and suggest that different chloroplast-nuclear signals might control different sets of genes.

S31

GENOMIC DISSECTION OF DROUGHT RESISTANCE IN WILD EMMER WHEAT

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Drought is a major environmental factor limiting crop yields worldwide. Wild emmer wheat (*Triticum turgidum* spp*. dicoccoides* (Körn.) Thell.), the allo-tetraploid (genome BBAA) progenitor of cultivated wheats, offers a valuable source of allelic diversity for various economically important traits including drought resistance. The utilization of wild emmer for wheat improvement requires a wide exploration of the wild germplasm and in depth understanding of the physiological, genetic and gnomic background of their adaptive mechanisms.
A total of 145 wild emmer accessions, consisting of 25 populations, and three control durum wheat cultivars were examined for drought responses under two irrigation regimes, well-watered control (~650 mm) and water-limited (~250 mm), as well as for microsatellite markers (both genomic- and transcribed- SSRs). A wide phenotypic diversity was found for productivity and drought related traits both between and more interestingly within populations, with a considerable number of accessions showing advantage in drought resistance over cultivated durum wheat genotypes. These findings corresponded with the wide SSR allelic diversity, of which 56% was found within populations and only 44% between populations. The greatest drought resistance capacity corresponded with the highest allelic diversity, both found in populations from intermediate aridity level (350-550 mm/year rainfall). Physiological responses to drought were further dissected by quantitative trait loci (QTLs) mapping of yield and drought related traits under two irrigation regimes. A genetic map was constructed using 152 F6 recombinant inbred lines, derived from a cross between wild emmer wheat from drought-prone environment and cultivated durum wheat. This map, consisting of 196 SSR and 491 DArT markers, accounted for 2317.1 cM with an average interval of 7.5 cM between markers. Major genomic regions controlling productivity, phenology and drought related physiological traits were identified. Several QTLs exhibited GxE interaction and accounted for productivity and related physiological traits under either the well watered or water-limited conditions. The identified genetic resources and QTLs are expected to facilitate the improvement of drought resistance in elite wheat cultivars by marker assisted breeding.

S32

DIFFERENTIAL GENE EXPRESSION OF WHEAT GENOTYPES AFTER FUSARIUM GRAMINEARUM INOCULATION ANALYZED THROUGH CDNA-AFLPS

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Fusarium head blight (FHB) is a devastating disease of wheat in many areas of the world. Molecular mapping projects led to the identification of two major FHB resistance QTL, FHB1 and Qfhs.ifa-5A. The actual function of these resistance genes is still unknown. We aim to identify expressed genes involved in the resistance reaction of wheat against FHB and to contribute to the functional clarification of the resistance reaction. Four wheat genotypes with contrasting phenotypes for FHB resistance due to the possession of the FHB resistance QTL were challenged with *Fusarium graminearum* or water. At 6 time points after inoculation (0h – 72h) differential gene expression was analysed by cDNA-AFLPs. Altered expression patterns after *Fusarium* inoculation were observed for 164 TDFs, corresponding to 3.4% of the analysed fragments. Most of these transcripts were up regulated after *Fusarium* inoculation and were unaffected by the analysed genotypes and their resistance level. 16 TDFs, 0.32% of the total analysed fragments, displayed differential expression after fungal attack depending on the genotype and the possession of the resistance QTL. Sequencing these TDFs revealed homology to wheat genes involved in metabolic pathway e.g. the phenylpropanoid pathway and glucosylation, but most are without a known function.

Poster abstracts

Session 4: WG3: Implementation of Genomics Approaches for Understanding Cereal Traits

P4.1

CHARACTERIZATION OF EPIGENETIC SILENCERS DURING SEED DEVELOPMENT IN BARLEY

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Seed development is regulated by epigenetic mechanisms as has been demonstrated by recent studies in Arabidopsis. The Arabidopsis FIE, MEA and FIS2 are parentally imprinted genes encoding Polycomb Group (PcG) proteins which play a role in the formation of a particular PcG complex that controls embryo and endosperm development. PcG complexes have been found to be associated with histone deacetylases (HDACs). In Arabidopsis, a particular type of HDAC belonging to the plant specific class HD2 and named HD2a has been found to play a role in seed development. Both PcG and HDAC proteins act as gene silencers mediating chromatin compaction and subsequently suppressing gene transcription. We have attempted to understand the epigenetic mechanisms regulating seed development in barley *Hordeum vulgar*e, a monocot crop plant of high economic value cultivated for its endosperm containing seeds. As a first step in this effort cDNAs coding for the FIE, MEA and FIS2 homologues as well as full length cDNAs for four HDACs were isolated and characterized from barley. Two homologues of the HD2 family (HvHDAC2-1 an HvHDAC2-2), and one homologue of each of the HDA1/RPD3 family, class I (HvHDAC1I-3) and class II (HvHDAC1II-1) were isolated, respectively. Expression analysis of barley PcG genes in different tissues and developmental stages showed that with the exception of two of the three FIS2 homologues examined, all genes are expressed in roots, shoot meristem, young shoots, leaves, stamens, unfertilized pistils and developing seeds 1-5 days after pollination but significant quantitative differences were observed in different tissues. Similarly, expression analysis of the four histone deacetylase genes demonstrated that they are expressed in all tissues and developmental stages. Furthermore, comparative expression analysis between two different barley cultivars that differ by almost 2.0 fold in seed weight was performed in order to unravel any correlation between PcG and HDAC gene expression and seed size.

P4.2

A FUNCTIONAL GENOMICS APPROACH TO STUDY A DROUGHT-RELATED GENE IN DURUM WHEAT

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Abiotic stresses and in particular drought have afflicted agriculture over the ages. Today, the importance of crop resistance to water stress, extremes of salinity, and harsh temperature is further increasing, in connection with both expanding extensive agricultural areas and increasing of extreme weather conditions due to global climate change. Many stress-inducible genes have been identified and insights into their functional roles in stress tolerance is gaining. This makes it feasible to improve crop stress tolerance by targeting stress-related genes either for genetic manipulation or for assisted breeding. We isolated and characterized the TdDRF1 gene codifying for a dehydration responsive factor in durum wheat. Quantitative RT-PCR was used to measure the expression profile of the three TdDRF1 transcripts in the following conditions: plant samples of different cultivars in time-course experiments were analysed, water stress experiments in greenhouse and stressed materials from controlled experimental fields at Obregon, CIMMYT (MX) were carried out. Tolerant and susceptible cultivars are analysed and the results from field samples and the greenhouse ones were compared.

P4.3

TOWARDS THE DISCOVERY OF GENE SPONSIBLE FOR ROOT HAIR ELONGATION IN BARLEY

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Root hairs are tubular outgrowths of root epidermis which extend root surface, take part in water and nutrients uptake, help to anchor plant in soil and interact with microorganisms. In our previous studies, several barley mutants with changes in root hair development were isolated after mutagenic treatment of barley varieties with N-nitroso-N-methyl urea (MNU) and sodium azide. Among them, one form characterized by short root hairs (rhs1.a) and additionally by short root system and dwarf phenotype was found. All three characters were controlled by separate recessive genes and linked to each other. The mutant was crossed with Steptoe and Morex varieties and F2 mapping populations were developed from both crosses. Using AFLP and SSR markers with the known position on barley maps, the rhs1 gene responsible for short root hair development was mapped on the chromosome 5H in the near-centromeric position. In order to isolate the gene of interest two approaches are used. The first one is aimed on the fine mapping of chromosomal region containing the rhs1 gene. For this purpose the Bulk Segregant Analysis (BSA) method and AFLP technique were used. Altogether 200 primer combinations from EcoRI/MseI and PstI/MseI restriction systems were screed in DNA pools. Twenty eight AFLP products segregated in bulks showing linkage with rhs1 gene. Up to date, the segregation of 10 AFLP markers was tested on 400 F2 plants from rhs1.a x Steptoe mapping population. The distance between markers and rhs1 locus ranged from 0.1 to 9 cM. In the closest neighborhood to rhs1 gene three markers are located: E38M62S134 (0.1 cM), E38M50S229 (0.5 cM) and E38M62S119 (1 cM). The segregation of these loci will be analysed on larger population. The BSA/AFLP method will be also used for fine mapping of genes responsible for short root and short stem development. The second approach relies on selection of possible candidate genes from rice, maize and Arabidopsis thaliana sequence data bases. Selected sequences will be used for isolation of homologous root hair genes in barley through the reverse genetic approach.

P4.4

RELATIONSHIPS BETWEEN HOMOEOLOGOUS REGULATORY AND STRUCTURAL GENES IN HEXAPLOID WHEAT TRITICUM AESTIVUM L. - STUDY ON FLAVONOID BISYNTHESIS GENES

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The flavonoid biosynthesis pathway leads to formation of phenolic compounds involved in many plant functions, including resistance to abiotic and biotic stresses. Structural genes participating in flavonoid biosynthesis pathway have been cloned and studied mainly in diploid plant species yet. Isolation them from hexaploid wheat *Triticum aestivum L.* (2n=6x=42, genome AABBDD) is complicated because of several homoeologous copies presence. In the current study three homoeologous and one non-homoeologous copies of a gene coding for flavanone 3-hydroxylase (F3H - one of the key enzymes of the flavonoid biosynthesis) were isolated and mapped in T. aestivum L. homoeologous groups 2 chromosomes. Specific PCR-primers to the individual copies of F3H gene were developed and used in quantitative RT-PCR on wheat lines carrying different homoeologous regulatory genes Rc. The genes Rc-A1, Rc-B1, Rc-D1 for anthocyanin coloration of wheat coleoptile were mapped in our earlier studies on chromosomes 7A, 7B and 7D, respectively, using SSR markers. Rc genes are considered to be regulatory genes for flavonoid biosynthesis pathway structural genes. From the results of quantitative RT-PCR, performed on cDNA obtained from each line during 5 days of the trait formation, several important observations were made. First of all, expression level of F3H mRNA was different uder control of different Rc genes, it was lowest under control of Rc-D1 and highest under Rc-A1, in absence of Rc (coleptile not colored) F3H mRNA was not expressed. We have also observed that from three homoeologous Rc genes Rc-D1 controls the most early expression of F3H mRNA, Rc-B1 – the most late. Non-homoeologous duplication of F3H gene on chromosome 2B was not expressed in presence of any Rc gene, only the homoeologous copies of these gene were expressed in wheat colored seedlings. Overall, we have concluded that input of homoeologous regulatory genes into differential expression of F3H mRNA is higher than those of homoeologous structural genes, and that no genome-specific relationships within regulatory and structural homoeologous genes exist. For financial support Elena Khlestkina thanks INTAS (young scientist fellowship, 04 - 83-3786), SB RAS (Lavrentjev young scientist grant, 111) and grant of the President of Russian Federation for young scientists (MK-566.2007.4).

P4.5

MOLECULAR MARKERS IN SEED PRODUCTION OF UKRAINE

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DNA-technology open new page in theory and practice of plant improvement. DNA-technology in plant breeding present as GMO and molecular markers. Researches in area of creation and detection GMO in Ukraine are not quite developed from the limited financing , while application of molecular markers did not have the substantial obstacles. Development technology of PCR-analysis created possibilities application molecular markers in wide scales, that allowed introduce its in practice of plant-breeding researches.In plant production of Ukraine molecular marker use in genetics, plant breeding and seed production. Molecular markers apply for estimation genetic variability, genotypes distribution accordingly to genetic distances, identification and registration varieties, creation databases of alleles composition selected loci of plant varieties. In genetics, this is clarification of phylogenetics relations between cultural species and their wild relatives – donors of agronomical important traits and for evaluation genetic relation between varieties. For such work multiloci dominants biallelic markers are recommended. In our researches the RAPD markers were used at first. Its were used for the analysis of interspecific polymorphism and clarification of phylogenetics relationship . Further in addition to RAPD more stable ISSR – PCR was introduced. ISSR-PCR was used by us in reconstruction origination wheat varieties of Belocerkovskaya 198 and Mironovskaya 264. These two phenotypically similar varieties from data of authors had different paternal forms. Results of ISSR-PCR analysis testify in behalf on that these heterozygous varieties possible had general parents. Cause particular interest relatively recently developed A.Schulman with co-workers in the Helsinki Biotechnological Center polyloci IRAP, where as a primers is used sequences from LTR and REMAP, where one praimer is microsatellite, and other is area from the long terminal fragments of retrotransposon. The strong criterion of reaction allows in both cases to get the well reproduced results. In our researches barley varieties of the Ukrainian breeding these variants of PCR analysis appeared very effective. The selection of primers detected polymorphism on the varieties of barley is conducted. Dendrogramm (TREES), that reflected phenogenetic relationship between investigational varieties of barley consists of two clusters. Spring varieties are located in the first cluster, and in the second are winter forms. Both systems of IRAP REMAP generate on principle similar dendrogramms, reflected pedigree. At the same time, as well as it was expected, rectifying REMAP polymorphism higher and consist 67.4% against 58.7% at IRAP. These polyloci systems allow to detect of intravarieties heterogeneity of the Ukrainian varieties of barley and wheat.In SPBC created DNA-typing data genotypes Ukrainean varieties wheat, barley, maize by SSRP-analysis, DNA technology is used in SPBC for marking of simple genes and QTLs controlled the important agriculture traits in particularly winter wheat flowering time, hardness/softness, maize fusariosis resistance, and wheat genotypes resistance to the phytopathogenes which is transferred from Aegilops cylindrica Host. With using

molecular markers for QTL brerders Plant Breeding and Genetics Institute (Odessa) with help biotechologists from South Plant Biotechnology Center create maize hybrid "Dialog".

P4.6

EVALUATION OF GERMPLASM COLLECTION AS A SOURCE OF VARIABILITY TO UNDERSTAND ABIOTIC STRESS RESISTANCE. APPLYING PROTEOMICS IN THE STUDY OF WHEAT ALUMINIUM TOLERANCE ON THE ARCHIPELAGO OF MADEIRA

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The Portuguese Archipelago of Madeira is located on the Atlantic Ocean, between latitudes 33º10 32º20 N and longitudes 16º10 17º20 W, 630 km west of the coast of North Africa. Since the beginning of colonisation, high crop diversity was established as a result of cultivar introductions from different geographical origins around the world, and their acclimatization to the island agro-ecological conditions. This crop diversity was maintained until today in traditional cropping systems, with farmers maintaining their own stock of seeds for generations. One of these crops was wheat. The selective pressure of the island widespread metal toxicity, with low soil pH and the aluminium presence in soil solution makes Madeira a good choice to search for new forms of aluminium resistance in this crop. This diversity is being preserved at the ISOPlexis Germplasm Bank (http://www.uma.pt/cem/isoplexis), including the one that concerns the resistance to aluminium. A biochemical and molecular characterization program is now underway to assess the preserved cultivars using protein polymorphism (storage proteins) and molecular markers (microsatellites). Over 50 wheat populations were screened for aluminium tolerance, using the erichrome cyanine staining test, root elongation and measurements of callose content in root tips. Seedlings were grown in hydroponics culture and submitted to very stringent Al conditions (100 and 200 µM). The obtained results seem to suggest that different tolerance mechanisms are present in the screened populations representing an opportunity for the identification of new sources of genetic variability. Seven cultivars representing a whole spectrum of responses to Al stress found on the island were chosen for selection, including Al resistant, semi-resistant and sensitive populations. Two parallel approaches to develop the model material were taken. In the first approach, five genotypes were selected either for resistance or sensitivity until the F3 generation. In the second approach, double haploid lines of four genotypes with different Al resistance were developed. In both approaches taken, Al resistance of the genotypes was assessed using the previously described screening tests as well as their performance is being evaluated in field trials. The selected genotypes will be used to study the genetics of Al resistance, and the mechanisms involved in responses to Al stress among the wheat cultivars. In order to achieve this goal, root and leaf protein expression analysis under Al stress, assessed by the 2D electrophoresis, will be conducted. We aim to use the proteomics approach to isolate proteins that co-segregate with aluminium tolerance in wheat roots. This will allow us to identify genes controlling Al tolerance, as well as to characterize some cellular mechanisms involved in aluminium resistance. We also intend to perform

molecular screening aimed at determination of QTLs involved in the response to aluminium in these populations. The possibility of using micro arrays to search for differential gene expression in sensitive and tolerant lines is also being considered. The

diversity in responses to aluminium stress among the Madeiran wheat genotypes suggests the possibility of finding new sources of genes that would be essential to the process of improvement of quality and productivity of the most commonly used crop species.

P4.7

PROTEOMICS ANAD PROTEIN FOLDING IN DEVELOPINF WHEAT SEEDS

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Storage proteins are pivotal in determining rheological properties of dough but their synthesis can be detrimentally affected by environmental factors. As the endoplasmic reticulum (ER) is the site of storage protein synthesis, it is believed that a number of resident ER proteins play a key role in dough quality. Two dimensional gel electrophoresis and tandem-MS analysis of the proteome of developing wheat seeds identified various proteins localised in ER including protein disulphide isomerase (PDI) and calreticulin (CRT). Peptide sequencing of PDI confirmed, for the first time, expression of the three isozymes encoded on the A, B, and D genomes in developing wheat seeds. Furthermore, two isozymes of CRT which were not hitherto characterised in wheat seeds were also identified. Both CRT isozymes are members of the CRT1/CRT2 group. CRT and PDI appear in different multiple forms at 10 dpa and 25 dpa suggestive of post-translational modification of these proteins during seed development. Further investigation of post-translational modifications and regulation of the different protein isozymes may aid in identifying the molecular mechanisms by which environmental factors influence dough quality. This work is supported by a Large Multi-Disciplinary Research Network Grant from the Danish Ministry of Science Technology and Innovation.

P4.8

DIFFERENTIAL GENE EXPRESSION BETWEEN WILD EMMER WHEAT GENOTYPES CONTRASTING IN DROUGHT TOLERANCE

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Plants respond and adapt to drought at the molecular, cellular and whole plant levels by activating a range of physiological and biochemical responses. These processes are controlled by a network of genes with diverse functions, which could be activated or repressed in response to drought stress. Drought is the most important single environmental stress limiting plant development and crop productivity of cultivated wheat species. Wild emmer wheat (*Triticum dicoccoides*), the progenitor of cultivated wheat, is a promising source for improvement of drought resistance in cultivated wheat. Microarray technology is an important tool for unraveling the molecular genetic basis of plant reaction to abiotic and biotic stresses. In the current study, we describe a comparison of global gene expression between drought resistant and drought susceptible genotypes of wild emmer wheat, under normal irrigation vs. drought stress conditions, using Affymetrix GeneChip® technology. This comparison showed that of the 61,127 probe sets, a total of 6679 transcripts showed a significant effect ($p<0.0001$) of stress/control, genotype, or stress×genotype interaction, in at least one of the four genotype/treatment combinations. Of them, 1121 transcripts showed more than 1.5 fold change from the average. Further analysis showed that 435 of these transcripts were differentially expressed between the two genotypes under drought stress. Of them, a group of 60 genotype-specific transcripts were highly expressed under drought stress in the resistant genotype. Gene annotation has shown that this group is involved in important biological pathways, such as: membrane structure, transport, carbohydrate and amino acid metabolism, DNA binding, senescence, and drought/cold response. Furthermore, some of these genes are known to be involved in drought tolerance. Therefore, this group is considered as a promising source for potential drought resistance candidate genes. Our results show that comparison of gene expression patterns between tolerant vs. sensitive genotypes is a promising approach for identification of candidate genes that may contribute to drought tolerance in wheat.

P4.9

UNDERSTANDING THE PARTIAL RESISTANCE CONFERRED BY THE PCH1 GENE TO OCULIMACULA YALLUNDAE IN WHEAT : MICROSCOPIC AND MOLECULAR APPROACHES

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Cereal crops in many parts of the world are often infected by fungi which attack the base of plants. One of the most important of these stem base diseases in wheat is eyespot, caused by two closely-related species of *Oculimacula*. These pathogens can cause severe damages on wheat crops, affecting both yield and quality of grains. Because *Oculimacula* sp. can easily adapt to fungicides, genetic resistance appears to be the most efficient way to control eyespot disease. An improved understanding of genetics and mechanisms of resistance to *Oculimacula* sp. is essential to devise more sustainable approaches to eyespot control. This project focuses on the Pch1 gene, which is a major gene conferring partial resistance to *O. yallundae*. This gene, introduced from *Aegilops ventricosa* into wheat, is the most efficient resistance gene and the most widely used by wheat breeders. The objectives of this project are to identify the defence mechanisms involved in a partial resistance conferred by the Pch1 gene and to isolate the Pch1 gene. First, we characterized the interaction between a wheat line carrying the Pch1 gene and *O. yallundae*. We established a relationship between symptom development and the changes occurring in pathogen development and plant responses during the interaction between a partially resistant wheat line carrying Pch1 and *O. yallundae*. To do this, we studied simultaneously the timing of symptom development, pathogen development and histological and molecular plant responses in eyespot inoculated and mock-inoculated partially resistant wheat. During the asymptomatic phase corresponding to coleoptile colonisation, local H2O2 generation and callose deposition were observed and PR genes were up-regulated. During the symptomatic phase beginning with the first leaf sheath attack, natural browning occurred in epidermal cells and the PR genes were more strongly up-regulated. These results show that changes in the pathogen development and plant responses occurred during the transition period between the asymptomatic to the symptomatic phases. Second, we initiated the map-based cloning of Pch1 in a population of 135 recombinant-inbred lines derived from a cross between *Ae. ventricosa* 10 (partially resistant, Pch1) x *Ae. ventricosa* 7 (moderately susceptible). The Pch1 gene is located at the distal part of the long arm of chromosome 7D. Using Bulk Segregant Analysis, a total of 28 AFLP markers were identified close to the Pch1 gene. Our present work focuses on the development of codominant PCR-based markers for the fine mapping of Pch1 in a large *Ae. ventricosa* population. This includes the development of STS (sequence tagged site) markers from AFLP markers and the use of the synteny between *Poaceae* and rice to derive markers from wheat EST sequences or directly from rice gene sequences. Future work will focus on the identification of defence mechanisms

involved in the partial resistance controlled by the Pch1 gene during the asymptomatic and symptomatic phases. To carry out this study, we developed isogenic lines for the

Pch1 gene and we will use a combination of 'omics' approaches (transcriptomic arrays, metabolomics and proteomics).

P4.10

DEK1 FUNCTION TO KEEP POSITIONAL IDENTITY IN THE SHOOT APICAL MERISTEM, VASCULATURE AND EPIDERMAL LAYERS?

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The single copy *Dek1* sequence is highly conserved between species. Discussion of putative functional differences from DEK1 aligment between all interesting and available sequences will bee presented together with our current model on DEK1 function. Due to the relatively simple structure of the endosperm with four cell types only; aleurone, starchy endosperm, transfer and embryo surrounding cells, it represents an excellent system to dissect molecular and cellular mechanisms of differentiation and development. Aleurone cell differentiation is of special interest due to its genetic effect on epidermal and L1 identity in general. Three genes are known to influence differentiation of the maize aleurone layer; *Cr4*, *Dek1* and *Sal1*, and their geneproducts colocalize in endosomes (Tian et al. in press). *Dek1* encodes a 240 kDa protein and comprises a predicted membrane spanning region (DEK1-MEM) containing a possible external loop (of importance to regulation), and a cytosolic calpain-like cysteine proteinase region (DEK1-CALP). *Arabidopsis dek1* T-DNA insertion lines, partly or completely lack aleurone cells, and display unorganized embryo development with irregular mitotic divisions in the outer layers in the embryo proper and in the suspensor. Embryo development is arrested at the globular stage, before the meristematic boundaries of the embryo are laid down. *Arabidopsis dek1* RNAi lines are not embryo lethal, however, they are heavily affected in the shoot apical meristem region (SAM), and grow to produce cotyledons and a few additional fused organs with pin-like leaf structures before growth arrest. The current model for aleurone cell specification involves DEK1 acting as a sensor for positional information, signaling to the cells where in the organism they are located, surface or internal. In this model DEK1 responds to a positional signal triggering aleurone fate, CR4 acts to counteract lateral inhibition of aleurone cell fate between aleurone cells, and proper concentration of DEK1 and CR4 in plasma membranes is maintained by internalization and degradation via trafficking through SAL1-positive endosomes.

We would like to present how, despite a functional microtubule apparatus, the *dek1* embryos have asymmetrical division planes, misplaced phragmoplasts and they appear tiered with possible protodermal defects. *WUS* and *STM* expression domains are increased by knocking out *Dek1*, while *ATML1*, *AP2*, *CLV*, *WOX* and *YABBY* seem unaffected. Interestingly in *dek1* knock-out and knock-downs, this increased number of cells expressing *WUS* and *STM* is combined with a flat, arrested shoot meristem. Finally preliminary data from using the glucocorticoid inducible GAL4 system to create tissue specific *dek1* knock-down lines in *Arabidopsis* supporting our model on DEK1 action will bee presented together with the mutant phenotype of the barley defective seed5 mutation.

P4.11

Molecular genetics for key agronomical traits in durum wheat: identification of genes of interest, functional analysis and molecular markers assisted selection

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The research group of the C.R.A.-Experimental Institute for Cereal Research of Foggia is focused on the study of molecular and genetic bases of key traits for the adaptation of durum wheat in Mediterranean environments. The activities involve the study of genetic diversity for yield capacity in stressed and non stressed environments, the mapping of important agronomic traits (abiotic stress tolerance, disease resistance and quality) to identify genomic regions containing loci/QTLs by means of linkage and association mapping as well as the isolation and the functional characterization of genes involved in abiotic stress response and grain quality traits. A collection of about four hundred primer pairs for microsatellites with known position is routinely utilised for the molecular characterization of genetic materials. We are constructing 3 linkage maps for traits linked to water use efficiency, gluten quality and resistance to fungal and virus pathogens. In particular, a linkage map with about 350 markers is available for resistance to leaf rust. Additional segregating populations are under development for several quality-related traits: gluten index, protein content, semolina colour and lipoxygenase activity. The overall strategy is focused to identify loci of interest and to develop a marker assisted selection for MAS breeding programs. A high-throughput platform dedicated to molecular markers (microsatellites and SNPs) will be available in the next months.

The work is supported by the following main projects:
FRUMISIS (Analisi del genoma del frumento duro per l'identificazione di geni utili al miglioramento della tolleranza a carenze idriche e alla salinità - Analysis of durum wheat genome for the identification of useful genes for improvement of tolerance to drought and salt stress) and AGRONANOTECH (Nuove tecnologie molecolari applicate al miglioramento genetico di specie di interesse agrario – New molecular technologies applied to breeding of crops), funded by Ministry of Agriculture of Italy.
AGROGEN (Laboratorio di GENomica per caratteri di importanza AGROnomica in frumento duro: identificazione di geni utili, analisi funzionale e selezione assistita con marcatori molecolari per lo sviluppo della filiera sementiera nazionale – Genomics laboratory for key agronomical traits in durum wheat: identification of genes of interest, functional analysis and molecular markers assisted selection for supporting the Italian seed companies) funded by the Italian Ministry of University and Research.

Speakers' abstracts

Session 5: WG4 Functional Genomics for Testing and Validation of Candidate Genes (FuncGen)

S33

MAPPING AND MARKER DEVELOPMENT OF DIFFERENT SCALD (*RHYNCHOSPORIUM SECALIS*) RESISTANCE GENES IN BARLEY

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Rhynchosporium secalis (*Oudem*) J.J. Davis, the causal agent of leaf scald is one of the major leaf diseases of barley (*Hordeum vulgare L.*). A number of resistance genes against the fungus were published up to now. The knowledge of inheritance and physical localisation of the major resistance genes enables the design of diagnostic markers and straightforward breeding strategies in barley. According to Patil 2001 and Björnstad et al. 2002, 15 major resistance genes or alleles against the fungus R. secalis have been described in barley so far, but not all of them have been mapped to a specific locus on the chromosome. Further resistance genes have been detected in genotypes of wild barley (*H. vulgare* ssp*. spontaneum*). The resistance genes for *Rhynchosporium secalis* were mapped on chromosome 1H, 3H, 4H, 6H and 7H (e.g. Björnstad et al. 2001, 2002; Genger 2000, 2005) and consecutively numbered up to Rrs16Hb. The last one was mapped by Pickering et al. 2006 on chr. 4HS. The nomenclature of the *R. secalis* resistance genes has to be transposed to the more correct Rrs/rrs system and to respective differential genotypes (for example: Rrs2Atlas for Rh2) in accordance to Björnstad et al. 2001, 2002. But there is still disorder in nomenclature because of missing test isolates, diverse reference genotypes (resistance donors) and diverse molecular markers in each map. So independent mapped resistance genes and QTL may sometimes be identical or allelic. In more detail, I want to give an overview over the breeding material and scald assay facility at our institute with respect to different scald resistance donors, DH mapping populations and mapping results as well as the production of NILs regarding to a new and major scald resistance loci on chr. 2H (Schweizer et al. 2004) and known loci on chr. 3H (Rrs1) and chr. 7H (Rrs2). Concerning the different NILs, we are looking for cooperation or funding to perform differential gene expression analysis.

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S34

CHARACTERIZATION OF GENES INVOLVED IN HOMOLOGOUS RECOMBINATION IN TRITICEAE. TOWARDS THE CHARACTERIZATION OF THE GENES INVOLVED IN GENE TARGETING IN TRITICUM

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The major goal of plant biotechnology is to develop new and improve existing elite cultivars. To meet this goal, it will be necessary to both improve existing and develop novel strategies for plant genome manipulation. The in situ modification of a resident gene or the insertion of a transgen in a controlled manner at a specific genomic position via homologous recombination is generally known as "gene targeting"(GT). The homologous recombination is the main mode of DNA integration in bacteria and lower eukaryotes. However, in higher eukaryotes including plants DNA integrates in the genome mainly by illegitimate recombination in a sequence-independent way. In plants, the targeting frequencies are very low. Hohn and Puchta (1999) reported frequencies of about 10-3 to 10-5.

An important aspect in the study of homologous recombination is the analysis of genetic systems that take part in the process. The gene Mre11 is a key actor in different recombination reactions namely in meiotic and illegitimate recombination. It acts in a complex named MRN with two other proteins, RAD50 and NBS1 (D'Amours and Jackson, 2002). The fact that Arabidopsis contains a single homologue of Mre11 strongly suggests that the major recombination pathways are conserved between eukaryotes. This kind of studies has been carried out in model species but little is known about homologous recombination (HR) in crops of economic interest like wheat. We are working in the isolation, molecular characterization and expression analysis of the genes involved in the HR process: Mre11, Rad50 and NBS1 in *Triticum* (genomes A, B and D).

The three genes are present in a single copy in each one of the genomes of wheat and are very conservative. The degree of homology for each gene was of about 97 and 98% among the three genomes of wheat.

Analysis of expression using the quantitative PCR technique showed different levels of expression for each gene in the species analysed.

The two-hybrid approach was used to verify the interactions between proteins MRE11-RAD50, as well as to observe the formation of homo-dimmers MRE11-MRE11 and RAD50-RAD50. Two hybrid analyses including NBS1 gene are in progress.

The interaction of proteins MRE11-RAD50 and MRE11-MRE11 was confirmed occurring crossed interactions between proteins of different genes with independence of the genome. Nevertheless the homo-dimmers RAD50-RAD50 have been not detected in the conditions of these experiments, maybe due to the requirement of a previous step like the interaction of RAD50 with MRE11 or a specific conformational structure of RAD50.

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S35

FUNCTIONAL ANALYSIS OF ANTIFUNGAL ACTIVITIES IN A WHEAT MULTIDOMAIN CYSTATIN TAMDC1

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A cDNA encoding a 23 kDa wheat multidomain cystatin TaMDC1 was cloned from cold acclimated winter wheat. Northern and western blot analyses showed elevated expression of TaMDC1 mRNA and protein during cold acclimation. Previously we have shown that the recombinant protein inhibits in vitro growth of phytopathogenic fungus Microdochium nivale causing the snow mold disease. TaMDC1 protein contains highly conserved N-terminal cystatin domain D1 and a C-terminal cystatin-like domain D2. Recombinant polypeptides containing each one of the D1 or D2 domain were separately purified and their papain inhibition activity and antifungal effect were analyzed. Both N-terminal and C-terminal recombinant polypeptides equally inhibited in vitro growth of M. nivale, while only the N-terminal polypeptide containing cystatin domain D1 showed CPI activity against papain. The second antifungal activity was localized in a peptide, called D2S, comprising of 50 amino acids at the N-terminus of D2. Site directed mutagenesis of D2S suggested that both 3D structure and amino acid sequence contribute to the antifungal activity.

S36

MECHANISMS OF PLANTS CONFERRING RESISTANCE TO XENOBIOTICS

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We are interested in mechanisms of plants conferring resistance to xenobiotics, in particular toxic metabolites produced by plant pathogens such as *Fusarium*. Our current working hypothesis is that necrotrophic fungal pathogens that do not fit into the gene-for-gene concept produce secondary metabolites (= small molecule effectors) which are able to suppress the defense response of plants or actively promote plant cell death. Based on bioinformatic analysis of sequenced fungal genomes, such pathogens most likely produce multiple compounds in planta, most of which are unknown and unstudied. So far most of the attention was devoted to compounds that accumulate in infected plants to levels that are toxic to humans or animals (mycotoxins).
On the side of the host plant the resistance against xenobiotics seems to be determined by proteins encoded by large gene families, which act at different levels, leading to complex quantitative genetics of resistance. We have previously studied ABC transporters of the PDR class (mediating drug efflux across the plasma membrane) and UDP-glucosyltransferases (UGT, inactivating toxins by formation of glucose conjugates), and glutathione-S-transferases (GSTs). The analysis of theses genes (e.g. by SNP marker development) is very difficult due to the large size of the gene families. Arabidopsis for instance has 15 PDR genes and more than 100 UGTs, and the gene family size is even larger in grasses. The intended research is to construct full length cDNAs of candidate wheat (or *Brachypodium*) genes and to perform functional tests using toxin sensitive yeast strains with multiple inactivated yeast pdr genes. We previously have focused on (clustered) UGT genes in Arabidopsis encoding enzymes that are able to inactivate the Fusarium toxins deoxynivalenol and zearalenone. The conclusion of this work is that even highly similar genes within a cluster can have very different substrate specificity, and also inducibility is not a good predictor of enzymatic properties. We intend to (systematically) test individual UGTs from wheat by heterologous expression in yeast to identify genes relevant for resistance breeding or biotechnological approaches.

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S37

APPLICATION OF TISSUE CULTURE AND BIOTECHNOLOGY METHODS FOR PLANT BREEDING

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Our group activities are focus on the in vitro culture and regeneration methodologies of cereals. These techniques may play an important role, shortening time needed for breeding program by double haploid (DH) production or introducing new variability by genetic transformation or mutagenesis. During the last years we have been working on the establishment of efficient protocols for DH production by anther and microspore culture in barley, bread and durum wheat, barley transformation and the combination of microspore mutagenesis with in vitro selection. At the same time, a programme for study the genetic control and the transcriptional variation associated to microspore embryogenesis was initiated in barley. Great efforts were made for the development of a transformation method in agronomical important cultivars of barley. Particle bombardment of immature embryos with bar and sacB genes was performed, and a total number of twenty transgenic lines from cvs Clarine and Golden Promise were obtained. Insertion of transgenes led to an increase in the level of ploidy in 4 transgenic lines, all of them showing fertility problems. Eleven transgenic lines transmitted at least one of the transgenes to the progeny. Morphological characterization has been performed in order to study the effect of transgenes insertion on agronomical important characters in T0 and T1 generations.

S38

HIGH-THROUGHPUT AGROBACTERIUM-MEDIATED TRANSFORMATION OF BARLEY

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Transformation of barley using an *Agrobacterium*-mediated system offers a number of advantages over biolistic-based methods in terms of efficiency and quality of the transgenic plants produced. Recent work in our group has improved transformation efficiencies from the 1-5% range to an average efficiency of 25%. Thus transformation efficiency is no longer a limiting factor to testing gene function in barley and it is now possible to consider developing a range of functional genomics resources in barley. Transgene expression can be unpredictable and unstable over generations. This is often linked to so called 'position effects' due to the genomic location of the transgene. We have addressed this issue in two ways. Firstly we have carried out detailed analysis of the transgene insertion site in barley. Secondly we have examined the effect of introns on both expression level and stability of expression of transgenes in wheat and barley. This work has recently led to significant increases in transgene expression levels. Transformation resources are made available through the BRACT (Biotechnology Resources for Arable Crop Transformation) project (www.bract.org). BRACT provides highly efficient transformation methodology for barley and other crops, a range of transformation constructs, training, advice and a full transformation service operated on a cost recovery basis.

S39

REGULATORY SEQUENCES FOR DEFINING TRANSGENE EXPRESSION IN WHEAT

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The use of genetic manipulation as a research tool to study gene function is well established in model plants such as arabidopsis and tobacco, and now that robust transformation systems are available, is becoming more widely used in wheat. At a practical level, a major bottleneck for using transgenic approaches in wheat has been the lack of well-characterised promoters for driving, tissue-specific, developmentally-regulated or inducible gene expression. In addition, there is a lack of basic, underpinning knowledge about how to predictably regulate transgene expression and the relative influence of enhancers, the core and proximal promoter regions, 5' and 3' utrs, introns etc. in an effort to address this we have begun a programme of systematic analysis of promoters using the gusa gene as a reporter in transgenic wheat. So far, we have focused our efforts on promoters that confer expression patters that are generally constitutive or that show seed-specificity. We have made transgenic wheat plants with over twenty different promoter: gusa cassettes and are making good progress characterising their expression profiles. Data on four generally constitutive promoters, whose detailed expression profiles differ, will be presented, along with several endosperm- or aleurone-specific promoters. Future work will include the modification of responsive elements and other key features of promoters and the analysis of altered expression profiles.

S40

THE WHEAT TILLING TO IDENTIFY NOVEL ALLELES OF CANDIDATE GENES

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We have established TILLING in wheat for functional validation of candidate genes. EMS-mutagenised populations were prepared from diploid (*T. monococcum*, *accession MDR308*), tetraploid (*T. turgidum durum cv. Cham1*) and hexaploid (bread) wheat (*T. aestivum cv. Cadenza*). Leaf material for DNA isolation was collected from M2 seedlings and M3 seed archived. These populations are being stabilised by single-seed descent and bulked for phenotyping in the field. TILLING was established using Cel1-digestion of annealed PCR products followed by separation on Licor gels. A number of candidate genes have been screened and numerous mutations identified. The hexaploid EMS population was shown to have a very high density of mutations, up to 50 per megabase of DNA, illustrating the efficiency of mutation screening in polyploid species.

S41

TILLMORE: A TILLING RESOURCE IN BARLEY ('MOREX' CV)

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Targeting-induced local lesions in genomes (TILLING) is a reverse-genetics approach providing an allelic series of induced point mutations from a population of mutagenized individuals (McCallum et al. 2000). At DiSTA (University of Bologna), a sodium azide-mutagenized barley (cv. Morex) population of ca. 5,000 M3 families, named TILLMore, has been developed for identifying mutants at target genes using the TILLING procedure.
Until now the TILLMore resource has been screened for several genes based on the screening of 8- to 12-fold DNA pools produced from M2 or M3 DNA samples, using LiCor and ABI3730 sequence analyzers. We have identified an average of 7 alleles per gene corresponding to an extrapolated rate of one mutation every 504 kb. Almost all the mutations observed were CG-TA transitions and several of them were missense, implying a change in amino acid sequence and therefore potentially affecting protein functionality.
Although TILLMore has been developed for reverse-genetics purposes, it is suitable for forward genetics analysis too. A phenotypic screening based on a field-grown trial (M3 families) showed a high percentage of families (ca. 33%) with visible phenotypes putatively derived from mutations. A preliminary screening of root phenotypes at the seedling level also showed that ca. 7% of families had some degree of altered root morphology. M4 seeds have been collected and properly stored. In order to enable the utilization of the TILLMore resource by the barley and cereal genetics community, a web-based central facility and a database on phenotypic information have been established (http://www.unibo.distagenomics/TILLMore).

Poster abstracts

Session 5: WG4 Functional Genomics for Testing and Validation of Candidate Genes (FuncGen)

P5.1

REPETITIVE-RELATED SEQUENCE REARRANGEMENTS IN TRITICALE: AN EFFECTIVE TOOL TO REVEAL INCREASED DIVERSITY

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Polyploidization is an evolutionary process in which hybridization and chromosome doubling induce enormous genomic stress and restructuring. To uncover polyploidization induced genomic diversity in the polyploid triticale, PCR-based molecular marker techniques involving retrotransposons and microsatellites were used, namely Inter Retrotransposons Amplified Polymorphism (IRAP), Retrotransposons Microsatellite Amplified Polymorphism (REMAP) and Inter Simple Sequence Repeat (ISSR). The comparative analysis of the banding profiles between triticale and its parental species (wheat and rye) uncovered nearly 52% rearranged sequences - parental bands absent in triticale as well as novel bands observed exclusively in the polyploid. The majority of these modifications are due to the loss of DNA fragments, predominantly from rye origin. Sequence analysis of rearranged fragments showed these to be retrotransposon related as well as coding sequences. One of the fragments from rye absent in triticale, obtained by REMAP, revealed homology with hydroxyproline-rich glycoproteins (HRGP), a protein that belongs to a major family of inducible defence response proteins involved in natural resistance of plants to injury, disease and various stress conditions. Perhaps the absence of this sequence in triticale is somehow related to its lower level of hardiness when compared to rye. Conversely, wheat-specific band absent in triticale and obtained with IRAP showed significant homologies with copia-like retrotransposons previously identified in *T. monococcum*, *T. aestivum* and *O. sativa*, namely Claudia and Barbara. The characterization of this lost sequence suggests the occurrence of recombination events between partial sequences of single or multiple retroelements and/or families of retrotransposons. To unravel the main chromosome domains involved in genomic restructuring phenomenon induced by polyploidization, Fluorescent in situ hybridization (FISH) with REMAP products was performed. Retrotransposon and/or microsatellite flanking sequences showed to be distributed throughout all rye chromosomes, with preferential accumulation in the heterochromatic sub-telomeric regions. The molecular and cytogenetic data suggest that the majority of sequence loss observed in triticale can be attributable to rye heterochromatic domains. The alteration of these heterochromatic regions can moreover affect the expression patterns of the terminal gene-rich regions of Triticeae chromosomes through the modulation of epigenetic markers, high lightening the possible role of repetitive sequences like retrotransposons and microsatellites in chromosome stabilization and genome speciation. The molecular markers IRAP. REMAP and ISSR proved to be extremely valuable tools to assess the increased diversity induced by the combination of two distinct genomes in the hybrid nuclei and can be broadly used to evaluate the diversity pool that can be produced and explored in plant breeding programs.

P5.2

GENOMICS OF GLUTEN GENES: HOW TO OBTAIN WHEAT THAT IS SAFE FOR CELIAC DISEASE PATIENTS

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Celiac Disease (CD) is a common (1% of the human population) food-related intestinal disorder, caused by gluten proteins of wheat, barley and rye. T cell stimulatory epitopes have been identified in all major classes of gliadins and glutenins. Some gluten peptides are defined as 'CD-toxic' because of their ability to induce damage to the intestinal mucosa of CD patients. Other peptides are called 'immunogenic' as they stimulate HLA-DQ2 or -DQ8 restricted T-cells, either directly or after modification by tissue transglutaminase (tTG). 'Immunodominant' peptides cause a strong reaction in generally all patients.
Using genomic and EST sequence information and epitope-specific T-cells and antibodies, the frequency of epitope occurrence has been shown to vary across wheat cultivars and Triticum species . We are currently screening modern bread wheat varieties using antibodies. The results will be compared with variation in a wide set of bread wheat accessions. In order to enhance the screening efficiency, we designed a sequential screening protocol: only those varieties that do not contain toxic alpha-gliadin epitopes will be further screened for gamma-gliadin epitopes; in next rounds LMW and HMW epitopes will be screened for. Alongside we develop a DNA test that employs pyrosequencing to detect SNPs in alpha-gliadin epitopes. As the composition of gluten genes is different for each of the three genomes, genome-specific and/or epitope-modifying SNPs have been identified in alpha- and gamma-gliadins. We quantitatively determine the ratio of the SNPs using cDNA from developing kernels, thus taking into account differential amplification among the three genomic loci.

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FUNCTIONAL GENOMICS: TRANSCRIPTION, INTERACTIONS, DISEASE GENES

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MATHEMATICAL REPEATS AND THEIR USES FOR ANNOTATING PLANT GENOMES

USING MATHEMATICALLY-DEFINED REPEATS TO ANNOTATE THE MAIZE GENOME

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A substantial portion of the maize genome, estimated at 60-70%, is repetitive, comprising mostly retrotransposable elements that inserted within the past 6 million years. Such high repeat content will present challenges to the efficient sequencing, assembly, and annotation of the maize genome. Since there is no guarantee that current libraries of manually-curated repeats are comprehensive, one of the primary challenges will be to identify novel repeats as the project progresses. We investigated an approach based on mathematically defined repeats that provides user control over desired repeat thresholds and does not require a pre-established library. Using the 0.45 X whole genome shotgun sequence prepared by the DOE Joint Genome Institute as a relatively non-biased representation of the maize genome, we computed the frequency of all constituent k-mers. With this index, we annotated/masked sequenced BAC clones with respect to their repetitive content. We demonstrate that this method is highly effective at identifying sequences harboring known classes of retroelements and distinguishing these from low-copy genic regions regardless of the maize strain assessed. In addition to supporting BAC sequence annotation, the k-mer method can also be used to guide sequence finishing projects by focusing attention on relatively non-repetitive regions, quantify relative genomic complexity and repetitiveness across the grasses, and evaluate the information content available in short sequences likely to be available using the new sequencing technologies. This work was funded by the NSF/DOE/USDA Sequencing The Maize Genome project (NSF #0527192).

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