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**EWAC – The European Cereals  
Genetics Co-operative**

**EUCARPIA Cereals Section**

# **BOOK OF ABSTRACTS**

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**Session 1**  
**EWAC – The Story of Successful**  
**Cooperation**

## **The history of EWAC and whatever happened to wheat aneuploids?**

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A brief account of the early years of EWAC – how it came into being – the major players involved in the early days and the aims defined at the inaugural meeting in Cambridge in 1968. Some of the achievements will be touched upon as well as the disappointments. Today it is pleasing that cooperation is still a thriving feature of EWAC but things have moved on and the techniques now available for identifying genes of value in breeding future varieties have changed, molecular probes having supplanted the cytological markers that were the only tools available in earlier years. However, what has happened to all those cytogenetical stocks, particularly aneuploid series, which were painstakingly assembled many years ago? There were over 70 monosomic series listed at the 7<sup>th</sup> Wheat Genetics Symposium in Cambridge in 1988. Many of these were at the early stages of development but many also were well established. Are these now redundant, or do they still have some value? Indeed, are they still available? Introducing new genes from the wild relatives of wheat seems to have taken on a new lease of life despite the expectation that molecular genetic engineering would supplant it. However, whatever happened to those alloplasmic lines, involving a range of different cytoplasms, mostly developed in Japan and the US? Apart from their effect on male sterility and their potential use in developing hybrids, they have hardly featured within the European context despite their evident influence on many agronomic characters. Answers to these questions may be relevant to the EWAC of today.

**Forty years of cooperative research (EWAC) in Poland**

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Collaborative research of Institute of Plant Genetics, Breeding and Biotechnology with scientific centers across Europe was initiated by professor Danuta Miazga in early seventies of the 20th century. At first the research work comprised of the transfer of monosomics to polish wheat varieties ‘Grana’ and ‘Luna’ and the development of ‘Grana’ addition lines with incorporated complete chromosomes from ‘Dańkowskie Złote’ rye. Established lines were used in studies on genes localization and chromosomes substitution. The chromosomes structure in few wheat varieties were analyzed and differences in translocation numbers among varieties were revealed. In further research substitution lines were tested in Polish environmental conditions. In ‘Chinese Spring/Cappelle-Desprez’ substitution lines, the effect of chromosomes on protein level and the level of amino acids in the protein fractions was studied. In ‘Cappelle-Desprez/ Bezostaya’ genes influencing the physical characteristics of stalks in response to lodging were located.

In 1990 began the co-operative study with John Innes Centre on dwarfing genes in wheat. Isogenic lines ‘Maris Huntsman’, ‘Maris Widgeon’, ‘Mercia’ and ‘Bezostaya’ carrying genes in locus Rht-B1, Rht-D1, Rht8 and Rht12 were analyzed. Long-term research confirmed the possibility of utilizing dwarfing genes in environmental conditions of Poland. The most promising genes were Rht-B1b, Rht-D1b and Rht-B1e. Along gibberellic acid-insensitive dwarfing genes, bread wheat breeding programs in Poland utilize Rht8 gene, which was identified in 19 wheat varieties. Isogenic lines were also used in studies on the effect of chlormequat chloride (CCC) and ethephon on yield components and gene expression. Collaboration with John Innes Centre resulted in the study on series of recombination lines ‘Mercia’ carrying Ppd-A1, Ppd-B1 and Ppd-D1 genes in polish climate conditions. The study revealed that photoperiod insensitive genes accelerated ear emergence while yield components depended on the year of research.

Cooperation with Leibniz Institute (IPK) Gatersleben led to the identification of gibberellic acid-insensitive dwarfing genes in 8 barley varieties. In addition, the allelic variation in Vrn loci by means of STS-PCR markers was determined. Dominant Vrn-H2 alleles were identified in winter barley varieties, whereas recessive alleles were found in over a dozen of spring varieties. Moreover, in cooperation with Leibniz Institute in Gatersleben, lines carrying the segments of chromosomes from D genome were investigated. The lines significantly differed from the control ‘Chinese Spring’. In terms of heading the earliest were introgressive lines with 2D and 7D chromosome segments and the latest were 5D lines. The shortest were 1D, 6D and 7D lines. Value of other quantitative traits depended on the year of investigation.

As a result of research collaboration within EWAC among many research papers two postdoctoral dissertations four PhD dissertations and about forty Master theses were performed.



**EWAC - the past 25 years (1991 – 2015)**

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This presentation is a personal review about 25 years of co-operation on cereals research. A period between 1991 when the 8<sup>th</sup> EWAC Conference was held in Cordoba, the first one I did attend, and 2015 the year of the 16<sup>th</sup> EWAC Conference in Lublin will be considered. In 1991 already series of genetic stocks including monosomics, chromosome substitution lines, alloplasmic lines, single chromosome recombinant lines, introgression lines, isogenic lines etc. were available. We presented co-operative studies on the genetics of plant height including the investigation of pleiotropic effects of near isogenic *Rht* lines grown in the UK, Germany and Yugoslavia (now Serbia). The whole range of genetic stocks was used intensively to associate many qualitative and quantitative inherited traits to certain chromosomes, chromosome arms or introgressed segments. In early 1990s molecular marker techniques became available. The knowledge gathered from the stock investigations became very useful and was often the pre-requisite for a precise mapping on certain chromosomes or chromosome arms.

Today, classical cereal genetic stocks are supplemented by a huge number of genotyped mapping populations. Beside progenies of bi-parental crosses (doubled haploid lines, recombinant inbred lines, etc.) panels for association mapping were created. There is also enormous progress in the development of molecular marker systems. For wheat chips carrying 90,000 SNP markers are used as a matter of routine for genome wide association analyses.

Here we give examples for 25 years of successful co-operation within the frame of EWAC. Genetic studies for a range of different traits are presented.

## **QTL analysis of response to water deficit in D-genome introgression lines of bread wheat**

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Improving the tolerance of cultivated plants to drought is considered to be one of the most complicated tasks for breeding because of a polygenic inheritance and a strong environmental influence. Quantitative trait loci (QTL) mapping now is widely used for dissecting such traits on more simple genetic components which may be used for selection. The wild relatives of cultivated plants may serve as a source of allelic diversity for abiotic stress tolerance. Bread wheat is one of the staple food crops in the world. The diploid species *Aegilops tauschii*, donor of bread wheat genome D, growing in highlands and dry habitats may be used for this purpose. In this work, a set of introgression lines of bread wheat cv. Chinese Spring carrying the genotyped genome D fragments was used for mapping QTLs associated with adaptation of photosynthetic and stomatal leaf apparatus, plant biomass and antioxidant enzymes activity as well as their tolerance indices. Twelve main regions were detected on chromosomes 1D, 2D, 5D and 7D carrying clusters of QTLs. Most of them were associated with a reaction to water deficit. The largest number of loci was detected on chromosomes 2D and 7D. For example, in the region of the microsatellite marker *Xgwm 539* of chromosome 2D 9 QTLs were co-localized associated with the absolute values of stomatal conductance and biomass on drought and their tolerance indices. Positions of some revealed QTLs corresponds to earlier found loci for physiological traits in other mapping populations.

## Diversity within Bulgarian old bread wheat germplasm

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A collection of 60 historic ('old') wheat (*Triticum aestivum* L.) varieties released in Bulgaria from the beginning of last century up to the early 1970s as products of traditional farmers' selection and early breeding activities has been gradually assembled. Genetic diversity was studied in different sets of genotypes with regard to the molecular variability, phenotypic characteristics, plant height, earliness, agronomic traits, disease resistance and nitrogen use efficiency. The study of genetic variation and distinctiveness among 28 accessions as revealed by microsatellites showed high allelic richness (173 alleles at 25 loci on 14 chromosomes, 6.9 average number of alleles per locus), 0.68 average PIC, 49 unique alleles at 18 loci, and 30.9 % average variety heterogeneity. Among this germplasm, potential sources of good productive potential, lodging resistance and disease resistance were identified. The study of N efficiency in 21 accessions at two N fertilizer levels and two environments differing by soil characteristics and crop predecessor showed variation with respect to the efficiency and N responsiveness depending on the environment. This old germplasm had evolved from a broader gene pool and therefore is a valuable though yet underutilized resource for breeding purposes.

## **Session 2**

# **Genetic Diversity *vs.* Plant Breeding**

## Molecular evidences of 100 years selection in Plant Breeding and Genetics Institute

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Changes in genetic diversity and distribution of alleles frequency among wheat varieties, which have been developed during the last 100 years in PBGI, were detected by microsatellite markers. Wheat varieties were presented by local populations that have high level of heterogeneity at the beginning of the previous century, contrariwise some modern varieties are linear and resistant to biotic and abiotic stress. Extensive involving of the genotype Bezostaya 1 and carriers of dwarfing genes from CYMMIT and Bulgaria in breeding programs resulted in spreading the predominant alleles – *Ppd-D1a* photoperiod insensitive and *Rht-8c*, *Rht-B1b*, *Rht-D1b* dwarfing genes with the high frequency among the modern winter wheat varieties. We have not revealed high level genetic polymorphisms with *Gli-1* and *Glu-A3* markers, developed by Zhang et al. (2003), in comparison with genetic polymorphism, that were tested by electrophoresis of storage proteins among the modern varieties (Polyshchuk et al., 2010). The most varieties of PBGI have been classified as “hard” wheats with alleles *Pina-D1a*, *Pinb-D1b* and there were not found null-alleles of *Wx*-genes in genotypes of common-type varieties. Thus, now in breeding programs which are focused on the creation of varieties with special quality characteristics □ “soft” and with low amylose content, introgressive hybridization and foreign sources of particular alleles are often used.

## Genome-wide association study in an Italian bread wheat collection

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This work focuses on a *Triticum aestivum* collection of 184 landraces, old, and modern varieties summarizing the last hundred years of common wheat breeding in Italy.

The primary aim of the study was to characterize the diversity of this collection and empower on it genome wide association studies (GWAS) for traits of agronomic and agro-ecologic interest. Eighteen traits (sorted in morphologic, agronomic and agro-ecologic) were measured during four growing seasons in field and two growing seasons in green house. Large phenotypic variation for all the analysed traits was observed.

The collection was genotyped using the the Infinium 90K array (Illumina). After filtering for call rate and polymorphic positions, 23,329 SNPs resulted useful for population structure analysis. Clustering and ordination analysis showed that the collection did not have strong genetic structure. Nevertheless, the lines could be sorted in two subpopulations grouping landraces and old varieties, and modern lines, respectively. The linkage disequilibrium (LD) baseline in the collection is  $r^2=0.06$  (95th percentile of markers more than 50 cM apart). LD decays with genetic map distance to  $r^2=0.1$  within 8.5 cM on average. The rate of LD decay is different for different chromosomes.

GWAS analysis was performed with 18,387 SNPs having MAF > 0.05. Of these, 15,604 were mapped. We used compressed mixed linear models, which effectively control for population structure while maximizing the power to detect marker traits associations (MTA).

Our GWAS approach identified suggestive MTA linked to several traits, such as spike morphology, kernel colour, flag leaf morphology and tillering.

## Genetic diversity of hexaploid wheat in Belarus based on SNP genotyping

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The genetic diversity within 77 accessions of hexaploid wheat growing in Belarus under breeding program, were assayed for polymorphism in comparison with 17 cultivars, represented variety across 7 European countries. We used high-throughput array to evaluate 384 gene-associated SNPs from published Cavanagh et al. (2013). A total 331 SNPs were genotyped, representing variability of 97.6% loci. Base changes at SNP loci include 80.8% transition and 19.2% transversion. Observed frequency of minor allele (MAF) was shifted towards alleles with  $MAF > 0.25$ . Of the 331 markers, only 8 (2.4%) detected  $MAF \leq 0.05$ , and 236 (71.3%) had  $MAF \geq 0.2$ , which showed its high differentiating ability for tested wheat accessions.

Our results revealed some common and contrasting patterns of the haplotype diversity in winter and spring accessions, which suggest different selection forces possibly acting in specific regions of the wheat genome throughout breeding. By neighbor-joining method, 94 wheat accessions were grouped into two major groups with high intra-group diversity in accordance with its type of vegetation and geographic origin. Genetic structure of Belarusian population of wheat is similar to Russian and Ukrainian variety and essentially different from west-European varieties.

Used SNP set are helpful in differentiating unique germplasm accessions and further allow us to validate allele-trait associations for wheat improvement in Belarus by genome selection.

**A comparative study of grain and flour quality parameters among Russian bread wheat cultivars developed in different historical periods and their association with certain molecular markers**

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A set of spring bread wheat cultivars bred in Russia were earlier genotyped with 23 microsatellite markers. This set was studied for grain and flour quality traits during several years. Their phenotypic diversity was compared with genotypic diversity. According the ANOVA data the group of cultivars developed in 1960-2006 years (“modern” cultivars) possessed a higher particle size of flour, flour strength, dough stiffness and extensibility, thousand grain weight (TGW). The olden cultivars developed before 1960 year contained significantly more gluten content in grain. A correlation analysis was carried out between the frequency of occurrence of certain alleles of microsatellite loci and the values of studied quantitative traits. Highly significant correlations were found between the markers and all traits. As was showed earlier, two groups of cultivars differ in specific set of microsatellite alleles. These specific markers of olden cultivars not presented in the group of “modern” cultivars correlated with large and small particle size, high gluten content in grain, high stiffness and high extensibility. The specific for “modern” cultivars alleles correlated with a high TGW, high vitreousness, and high dough strength. The olden cultivars developed before 1960 year were more heterogeneous and may serve as donors of alleles enlarging the variability for quality traits.

The work was supported by the State Budget Program (Project No VI.53.1.3).



## **Cereals genomics and genomic selection – new era of modern cereals breeding**

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At the beginning of the 21st Century, mankind faces the dual challenges of providing enough food for a growing population with a background of reduced resources and a more variable climate. In this context, genomics and associated molecular marker technology must play an important role in developing new varieties better adapted to address these challenges. During the last decade, molecular marker technology has provided a wide range of novel approaches to improve selection strategies and together with the rapid accumulation of genomics tools and the emergence of high throughput technologies has facilitated practical implementation into cereal breeding programmes.

Genetic engineering allows the creation of gene combinations and other modifications not possible through classical breeding methods and when considered together with conventional and marker-assisted precision breeding technologies, provides a powerful set of tools for delivering the advances in efficiency we need. The complete restoration of pollen fertility and reduced ergot infection in hybrid rye and the enhancement of foliar disease resistances in wheat and barley are all prime examples where selection intensity and accuracy can be accelerated by the application of molecular marker technology.

The availability of new molecular tools and technologies is beginning to filter through the breeding programmes to have a significant impact on plant variety development and is proving to be the essential element required to accelerate this process. The results of specific applications of molecular markers, potential of genomic selection and the application of genomics and genetic engineering in cereals will be presented and discussed.

# **Session 3**

## **Trait Evaluation and Genetic Mapping**

## **Recent advance in research of flowering time genes and their effects at the CRI, Prague**

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Long term study of wheat flowering time (FT) genes has been continued in three main directions in last ten years: Search for new genes / alleles influencing FT, analyses of effects of the FT genes, and production of special genotypes (lines) for these genes.

Characterization of the, *QFt.CRI-3B.1*, probably *eps* gene, and its effects has been continued using two NIL mapping populations despite the problems with phenotyping and obtaining polymorphic markers due to a closeness of the gene region to the chromosome 3B centromere. This region is spanning a large genetic distance between markers *Xgwm285* and *Xcfa 2170*, and to achieve physical map of the gene, it is needed to get closer to the gene locus.

Crosses between two cultivars, Granny and Trappe, differing only with carrying alleles *Vrn-B1c* and *Vrn-B1a*, respectively, have been done aiming to judge effect of the recently described new allele *Vrn-B1c*.

Developmental study was done with a set of genotypes carrying different *Ppd-B1* alleles that influence photoperiod sensitivity. According to the Waddington scale, the rate of plant development decreased in the order: Kaerntner Frueher, Paragon (Sonora 64 2D), Paragon (GS-100 2A), Paragon (CS 2B) and Paragon (sensitive to SD). Strong effect on difference in earliness occurred within the SSD mapping population between spring wheat cultivars Kaerntner Frueher and Paragon, which could be ascribed to the dose effect of *Ppd-B1* alleles.

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007- 2013) and from Ministry of Agriculture project no. MZe RO-0414.

## **Importance of flowering time loci for NS wheat breeding program**

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Flowering time is of the great importance for wheat breeding and is crucial for adaptation to different climate zones. Likewise, fine-tuning of flowering time plays the key role in order to increase yield potential and stability of wheat genotypes. The objective of this study was to dissect genetic basis of flowering time in the set of genotypes from Core Collections of Small Grains Department, Novi Sad. Field experiments were conducted in randomized block design during ten growing seasons (2000-2009). All varieties were genotyped by microsatellite markers applying fragment analysis using genetic analyzer ABI3130. Population substructure was revealed by software Structure and Principal Coordinate Analysis (*PCoA*) based on SSR markers. The analysis of marker-trait associations was carried out by using Tassel v.2.1. program. The significant and stable associations were detected using two methods: general linear model and mixed linear model. A total of 7 markers were significant and common for both models. The QTL near marker *gwm11* located on 1B chromosome has shown the highest stability in 6 (out of 10) years investigated. The findings of new QTLs, beside well-known *Ppd* and *Vrn* genes, could have a great impact to shortening of flowering time, which has the preferences in our environmental conditions.

This research was funded by the EU via FP7 project ADAPTAWHEAT (project number: 289842) and by the Ministry of Education, Science and Technological development of Serbia (project number: TR31066).

## **Interactive effects of water and salt stress on wheat growth and productivity under greenhouse condition**

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The objective of the study was to use the HAS-Shoot Stress Diagnostic System to analyse salt tolerance of wheat genotypes under well watered and drought conditions. The experiment was conducted with 14 wheat (*Triticum aestivum* L.) cultivars, from Serbia (5), Austria (4) and Azerbaijan (5), which were chosen on the basis of data available for their salt and drought tolerance.

Plants were grown under 4 watering/salt conditions:

1. Well watered (60 % field capacity) and no salt (NaCl ) added (control 1),
2. Water limited (20 % field capacity) and no salt (NaCl ) added (control 2),
3. Well watered (60 % field capacity) and saline conditions (0.2% NaCl),
4. Water limited (20 % field capacity) and saline conditions (0.2% NaCl).

Various morphological, physiological, and biochemical parameters involved in salt and drought tolerance processes were investigated at different developmental stages. The results showed large difference among the studied cultivars, and revealed tolerant and sensitive genotypes. The best performance in total grain yield under salt stress alone was observed in the NS-Avangarda, Gobustan and Tale-38 cultivars, while under water stress alone the Gallio, Balkan and Grymzyl gul-1 showed the highest grain yield. Under conditions of combined water and salt stress the Capo, Tale-38, and NS-40S showed the best performance. Grain yield stability was also the highest in the Capo and Tale-38. The obtained results can help the breeders from three different geographic regions in the selection and crossing programs to achieve good level of drought/salt tolerance.

This research was funded by the Program of Transnational Access to European Plant Phenotyping Network (grant agreement no. 284443.) and by the Ministry of Education, Science and Technological development of Serbia (project number TR31066).

## Characterising *Rht8* in wheat: fine mapping & agronomy

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The Reduced height 8 (*Rht8*) semi-dwarfing gene in wheat decreases cell elongation in the two uppermost internodes to reduce overall height by 10% compared to wild-type. Most commercial wheat carries either the *Rht-B1b* or *Rht-D1b* semi-dwarfing genes, but these decrease seedling vigour in drought conditions. *Rht8* is an attractive alternative since it improves lodging resistance without yield penalty. *Rht8* was delimited to a 1.29 cM interval on chromosome 2DS, and lines containing *Rht8* were found to have a reduced response to brassinosteroids.

We have further fine-mapped *Rht8*, using mRNA-Seq of the parents to the fine-mapping population and also used bulk-segregant analysis. We also developed markers from the iSelect array, synteny and wheat 2DS sequence, and propose candidates for *Rht8*. We used the newly-developed markers to map a 2D-height QTL in a UK Avalon x Cadenza population to the same region on 2DS as *Rht8*.

We developed Near Isogenic Lines in the UK elite spring wheat, Paragon. These NILs were used to assess the agronomic impact of *Rht8* in UK field conditions, challenged by different irrigation and Nitrogen treatments. Preliminary analysis indicates that *Rht8* incurs a yield penalty under high-yield potential conditions, however the penalty is reduced under low N, non-irrigated conditions. A crossover interaction of increasing yield under low N was found in some trials.

Finally, we continued the work of Viktor Korzun and the late Tony Worland to introgress putative novel *Rht8* alleles into a common background. Initial results from the first generation of glasshouse-grown homozygous plants indicate no significant height effect from the gwm261 *Rht8* allele. However, a general trend was observed of a height increase in lines carrying the rare allele.

## **Molecular approach to validate the transfer of APR-*Lr* genes into Romanian adapted wheat genotypes**

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The continuous and unpredictable evolution of rust diseases, under global and local climate changes, emphasized the wheat breeding priorities, in this respect. In Romania, leaf rust (LR) caused by *Puccinia triticina* reemerged in the last years as the most frequent rust in wheat crop, causing yield losses. To date, 74 leaf rust resistance genes have been catalogued in wheat, but only a few of these are race non-specific and confer adult plant resistance (APR) quantitatively expressed in terms of *slow-rusting* and durability. The *Lr34*, *Lr46*, *Lr67* and *Lr68* genes with pleiotropic adult plant resistance (PAPR) to leaf rust, stripe rust, powdery mildew, and stem rust are the most studied.

The aim of this study was to validate the transfer and pyramiding of designated *Lr34*, *Lr46* and *Lr67* genes into the Romanian bread wheat adapted genotypes Glosa and Miranda, using molecular markers. Analysis of F2-F3 lines from crosses developed at NARDI Fundulea reveals the transfer of targeted *Lr* genes. 45 out of the 224 analysed genotypes presented the *Lr34* and *Lr46* genes and one genotype the *Lr34* and *Lr67* genes (Line 274-1).

Phenotypic distribution analysis of leaf rust severity and AUDPC, under field artificial inoculations, showed generally a higher resistance of *slow-rusting* type in carriers of these *Lr* genes pyramiding. Accordingly, the additional presence of other *Lr* genes, still unknown, could not be excluded.

Deployment of more APR *Lr* genes, validated by molecular markers will allow the successful development of pre-breeding Romanian bread wheat genotypes with improved durable resistance to leaf rust.

The present work was funded through the UEFISCDI ANCS (National Authority for Scientific Research) Research Project PCCA 99/2012: “Phenotypic and molecular approaches to develop durable adult plant (*slow-rusting*, race non-specific) resistance to leaf rust (*Puccinia triticina*) in wheat (*Triticum aestivum*)”.

**The study of interaction of genes for spike shape located in 5A chromosome**

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The gene  $Q^S$  determining elongated and loose spike shape as in *Triticum spelta* L. was found in a winter line 84/98<sup>w</sup> with introgression from *Ae. speltooides* and located in 5AL chromosome. The line was obtained on the genetic basis of spring wheat cv. Rodina by I. Lapochkina with co-workers. The spring near-isogenic line i: Rodina  $Q^S$ , the carrier of  $Q^S$  gene was developed. The gene  $C^{17648}$  determines compact spike shape as in *Triticum compactum* Host. It was transferred in a spring near isogenic line ANBW-5A of cv. Novosibirskaya 67 from the mutant line MA17648 of durum wheat by N. Watanabe with co-workers. They mapped it to 5AL chromosome. Genes  $Q^S$  и  $C^{17648}$  are dominant to the normal spike shape of *Triticum aestivum* L. The aim of the work was to determine the interaction of the genes located in the same chromosome arm. A genetic analysis of the trait in two crosses i: Rodina  $Q^S$  x ANBW-5A and 84/98<sup>w</sup> x ANBW-5A showed that the gene  $C^{17648}$  is epistatic to the gene  $Q^S$  and they are inherited independently. The linkage between the genes for growth habit and awned spike in the hybrid population involving the winter introgression line 84/98<sup>w</sup> was found again as earlier in other crosses with this line.

We are grateful to Dr. N. Watanabe for providing the seeds of near-isogenic line ANBW-5A.



# **Session 4**

## **Wheat Initiative**

**The Expert Working Group on Wheat Genetic Resources**

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# **Session 5**

## **Wide Crosses, Physiology and Adaptation**

**The studies conducted at interspecific and intergeneric hybrids in Institute of Plant Genetics, Breeding and Biotechnology at University of Life Sciences in Lublin**

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Interspecific and intergeneric hybridization is a very useful tool in the breeding of cultivated species from *Triticeae* tribe. This technique has been widely used to introduce desirable traits from wild relatives to cropped species and to increase the genetic variation amongst the species by developing new varieties. The breeding programs are looking for genetic sources of additional traits including resistance to disease, lodging or various unfavorable environmental conditions such as salt and drought tolerance, as well as starch profile. Wide crossing is also used to new genera development, one such example is Triticale, which has been obtained by cross wheat (*Triticum* ssp.) as the female parent and rye (*Secale* ssp.) as the male parent. However, as a synthetic crop it is not genetically as diverse as naturally evolved crops. Hence, the lack of inherent genetic diversity may be overcome by the varied spectrum of gene pool derived from wild species. The development of wide crosses will also enable to gain some knowledge about crops evolution by their resynthesis, that will be valuable base material in plant breeding.

In Institute of Plant Genetics, Breeding and Biotechnology many various interspecific and intergeneric hybrids have been obtained. To develop these hybrids many wild relatives have been used, inter alia, species from *Aegliops*, *Dasyphyrum* or *Agropyron* genera. Moreover, many primary triticales have been received by wide crossing employment, where various cultivars of spring and winter wheat and rye with desirable traits were used.

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

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Common wheat genome is plastic due to its allohexaploid nature and tolerates alien introgressions. This facilitates crop improvement by introducing of new genes from wild species. The introgression line 8.1 originates from a cross between wheat cv. Tähti and *Triticum militinae* and confers improved powdery mildew resistance. The major resistance locus was mapped to the long arm of chromosome 4A (4AL). Moreover, 4AL arm contains ancestral translocations from 5AL and 7BS chromosomes. This makes the 4AL arm an ideal model for studying chromosomal rearrangements. 4AL of cv. Chinese Spring (CS) and 4AL-TM of introgression line 8.1 were flow-sorted and sequenced. A consensus map of 624 DArT markers was used to construct a 4A-CS GenomeZipper composed of 2,237 virtually ordered genes. A stringent comparison between 4A-CS map and other chromosome arms of cv. Chinese Spring allowed precise characterization of ancestral translocations on 4AL with two distinct regions comprising 230 and 531 genes assigned to 5AL and 7BS, respectively. A similar approach was used to identify the *T. militinae* introgression at distal end of 4AL. In Chinese Spring, the specific region comprises 395 genes, from which only 132 orthologous genes were identified from *T. militinae*. This study demonstrates that coupling chromosome sorting and Next Generation Sequencing provides a powerful tool for structural genomics and identification and characterization of chromosomal rearrangements.

This work has been supported by the Operational Program Education for Competitiveness — European Social Fund (project CZ.1.07/2.3.00/20.0165), and by Estonian Ministry of Agriculture.

## Interspecific polymorphism at non-coding regions of chloroplast DNA in *Secale* species

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Several published universal primers for amplification of non-coding regions of chloroplast DNA were tested whether they could amplify respective regions in *Secale* species. Published universal primers for amplification of *atpB* - *rbcL* intron, *trnT* (UGU) - *trnL* (UAA)5' and *trnD*[tRNA-Asp(GUC)] - *trnT*[tRNA-Thr(GGU)] successfully amplified the respective regions in *Secale* species. However, the primers for amplification of chloroplast *trnK-trnK* and *trnL* (UAA) 3' exon - *trnF* (GAA) failed to amplify the respective region. The results showed that the investigated regions of chloroplast genome are variable in most of the tested taxa and contain multiple variable regions. These regions should serve as useful molecular markers in phylogenetic studies of closely related species, at least at the interspecific level in *Secale*.

**Analysis of gibberellins biosynthesis genes expression alteration in response to plant growth regulators application in barley (*Hordeum vulgare* L.) plants with different dwarfing genes**

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One of the most common problem in cereal crops is occurrence of the lodging. That process can lead to inhibition of plant development, changes in regular life function and contribute to economical losses by significant decrease in yield. Gibberellins as a plant hormones are responsible for plant growth and development.

The purpose of this study was analysis of growth regulator application (chlormequat chloride – CCC, ethephon and trinexapac-ethyl) influence on alteration of expression of the genes encoding gibberellins biosynthesis enzymes (*CPS*, *KS*, *GA2ox*, *GA20ox*, *GA3ox*) in barley (*Hordeum vulgare* L.). Analyzed plant material comprised genotypes with different dwarfing genes: Triumph with *sdw1*, Hv287 with *sdw3* and Falk Mutant with *Dwf2* gene. As a control, tall form without dwarfing genes (Morex) was used. For determination of analyzed dwarfing genes transcript level in plant tissue qPCR method based on SYBR Green dye was performed.

Obtained results indicate that application of growth regulators effects on the level of analyzed genes transcription in barley. In all tested lines the *CPS* and *KS* genes expression was enhanced after all three growth regulators treatment. Similar response was noticed for the *GA20ox* gene, where only in Falk lines the expression decreased. In other two cases (*GA3ox* and *GA2ox*) the reaction was dependent on tested dwarfing genes, however after CCC treatment lower expression in most cases was observed. Additionally, trinexapac-ethyl enhanced *GA2ox* expression in majority of analyzed barley forms.

**Effect of plant growth regulators on transcript levels of genes encoding enzymes involved in gibberellin biosynthesis pathway in common wheat isogenic lines with different *Rht* genes**

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The introduction of the dwarfing genes into wheat genetic background has become one of the most effective ways of defence against lodging. Another approach in lodging prevention is the application of plant growth regulators that inhibit gibberellin biosynthesis by blocking the activity of enzymes involved in the process. These gibberellin inhibitors include chlormequat chloride (CCC), ethephon and trinexapac-ethyl. The aim of study was the expression analysis of the genes encoding enzymes involved in gibberellin biosynthesis pathway in wheat with different dwarfing genes treated with aforementioned plant growth regulators.

Analysed plant material consisted of common wheat (*Triticum aestivum* L.) near isogenic lines of the 'Bezostaya 1' carrying *Rht-B1b*, *Rht-B1d*, *Rht-B1e* (gibberellic acid-insensitive) and *Rht12* (gibberellic acid-sensitive) dwarfing genes. The real-time PCR was used to determine transcript levels of *CPS*, *KS*, *GA20ox*, *GA3ox* and *GA2ox* genes.

The study revealed that application of plant growth regulators has an effect on the expression of genes involved in gibberellin biosynthesis. The expression profiles obtained for wheat plants treated with CCC and ethephon were similar in most cases, whereas plants subjected to trinexapac-ethyl revealed different transcript profiles in analysed wheat lines. *GA20ox* transcript level was significantly higher in all wheat lines treated with trinexapac-ethyl while CCC and ethephon caused an increase only in *Rht-B1d* line. CCC and ethephon decreased the expression of *GA2ox* while the effect of trinexapac-ethyl was opposite. All isogenic lines exhibited an increase in the transcript level of *CPS* in response to each of the three gibberellin inhibitors.



## **Genetic analysis of short term responses of Latvian barley populations to conventional vs organic farming systems**

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Climate change challenges breeders to maintain yield stability and resistance to biotic and abiotic stresses in the context of increasing climatic fluctuations. Most widely used crop varieties are genetically uniform and have no buffering capacity to respond to environmental fluctuations. In contrast, populations contain intra-population diversity, which can promote compensation and complementation so they can respond to natural selection pressures and adapt to their respective environments.

We investigated spring barley populations from two simple cross combinations: Primus/Idumeja and Anni/Dziugiai. Populations from each cross were cultivated and exposed to natural selection in both organic and conventional sites for 7 seasons.

DNA was extracted from 95 plants of each population cultivated in organic conditions and 96 plants from each of population cultivated in conventional conditions. Nine SSR markers were used to genotype the four populations.

In total, 65 alleles were amplified, 2-9 alleles for each locus. In populations derived from the cross Primus/Idumeja 44 alleles were detected in each population, although 9 private alleles were detected in the population cultivated in organic conditions, and 5 in the population cultivated in conventional conditions. In the population derived from the cross Anni/Dziugiai and cultivated in organic conditions, 38 alleles were detected, of which 9 were private, whereas in the population cultivated in conventional conditions 24 alleles were detected out of which 1 was a private allele.

The preliminary results obtained demonstrate that cultivation of the same spring barley population in different agro-ecological conditions, such as organic versus conventional, results in changes of intra-population diversity and cultivation in conventional conditions may lead to loss of genetic diversity.

# Poster Presentations

## **Genetic studies on seed longevity in wheat – artificial ageing vs. long term storage**

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Seed longevity is of particular importance for crop germplasm conservation but also for seed industry. It is dependent on environmental conditions during seed development and harvest as well as post-harvest storage conditions, mainly temperature and moisture content. However, there is also a genetic component determining the time of seed survival. In order to study the longevity of seeds in a reasonable period of time (not spending years) artificial ageing tests were established. Seeds are exposed for short periods to an increased temperature and relative humidity causing rapid seed weakening. The International Triticeae Mapping Initiative (ITMI) mapping population, derived from a cross between the variety ‘Opata 85’ and the synthetic hexaploid wheat ‘W7984’ was used in the present study. Recombinant inbred lines (RILs) of this mapping population were analysed after artificial ageing as well as after long term storage (10 °C/ 50% RH) for up to 14 years. RILs were reproduced either at experimental fields at IPK in Gatersleben, Germany or at the University of California Intermountain Research and Extension Center in Tulelake, northern California, USA. Four replicates of 50 seeds each were subjected to standard germination tests following the International Seed Testing Association rules and data obtained were used for QTL analysis. Significant loci were detected on different chromosomes of all three wheat genomes. Contradictory mapping positions confirmed that growing and/or storage/ageing conditions exert a large influence on seed germination activity.

**Development of a core set of single-locus EST-SSR markers for diversity assessments of foxtail millet (*Setaria italica* L.)**

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This study was carried out to identify EST-SSR marker alleles able to distinguish 804 foxtail millet collections from RDA genebank in Korea, and to validate the utility of the genotype specific EST SSR markers in genetic diversity and assessment of landraces purity. 66,027 ESTs sequences on foxtail millet (*Setaria italica* (L.) P. Beauv.) were download from the NCBI nucleotide database and 376 EST sequences containing SSR motif longer than 24 bp were selected to design the EST- SSR markers. 320 pairs of SSR primers were designed and used for validation of the amplification and assessment of the polymorphism. Finally, 22 polymorphic primer pairs were developed. One hundred thirty four alleles were detected and revealed by using 22 EST-SSR markers, from two to eight alleles with an average 6.1 alleles per locus in 804 foxtail millet accessions. The average value of gene diversity ( $H_E$ ) and polymorphism information content (PIC) for EST-SSR marker was 0.626 and 0.556 within populations, respectively. Our results showed the moderate level of the molecular diversity among the foxtail millet accessions from various origins. The phylogenetic tree represented three major groups of accessions were not correspond with geographical distribution patterns except a few cases. The lack of correlation between the clusters and their geographic location might considered to their different genotype. Our study will provide a better understanding of genetic relationships among various germplasm collections, and it could contribute to more efficient utilization of valuable genetic resources.

**Cumulative effects of Lr34, or genes, and 1AL/1RS translocation on some agronomic traits in a set of wheat mutant/ recombinant DH lines**

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Based on preliminary field observation, a set of 30 mutant/recombinant DH lines were tested for presence/absence of the Lr34 (leaf rust resistance gene), or (osmotic adjustment gene), and 1AL/1RS translocation.

The lines were developed by specific protocol using two winter wheat genotypes, two irradiation cycles and Zea system DH- technologies. Cultivar Izvor released in 2009 at NARDI- Fundulea has been characterized as drought tolerant genotype due to its high yield ability in droughty years, carrying or recessive allele on 7A chromosome. The second genotype, F0062834- G, an advanced breeding line, manifested good resistance to foliar pathogens, high yielding potential in areas without water stress and carries 1AL/1RS translocation.

The first mutagen treatment (200 Gy) was applied on the seeds of both genotypes. The second treatment with different doses was applied on hybrid seeds resulted from direct (100 Gy) and reciprocal crosses (200 Gy) of M<sub>1</sub> plants derived from the first cycle. Then the plants (M<sub>1</sub>) were crossed by maize under greenhouse conditions, haploid plants regenerated “in vitro”, colchicine treatment applied on plantlets and DH<sub>0</sub> seeds harvested.

A number of 30 DH lines, from more than 400 produced up to now, were selected for further tests according to preliminary field observation and presence / absence of the two gene and 1AR/1AR translocation.

The paper presents some results regarding the foliar disease resistance, plant physiological parameters and TKW.

## **Monosomic analysis of leaf hairiness in isogenic lines of bread wheat Novosibirskaya 67**

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Monosomic analysis was carried out in order to determine a chromosomal location of genes controlling leaf hairiness in the near-isogenic lines ANK-7A, ANK-7B and ANK-7C of bread wheat. The lines were obtained on the genetic basis of spring wheat cultivar Novosibirskaya 67 (N67). The recipient cultivar has a soft on touch and uniform hairiness on leaf surface while the lines carry long and tough trichomes inherited from two Chinese and one Soviet cultivars. Hairiness was studied on detached leaves using the method of high-throughput phenotyping LHDetect2 which allows separating the phenotypic classes on the basis of quantitative characteristics of leaf hairiness among segregates in crosses. It was found that chromosome 7B of all near-isogenic lines carry the gene determining the presence of long trichomes and chromosome 7D of cv. N67 carry the gene enhancing trichome density. The obtained data allowed formulating the hypothesis about the presence of homoeoallelic gene series controlling leaf hairiness in chromosomes of the seventh homoeological group of bread wheat and its relatives.

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**Characterization of HMW-GS alleles associated with D and B genome of wheat *Triticum aestivum* L.**

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Bread wheat quality is mainly correlated with high molecular weight of glutenin subunits (HMW-GS) of endosperm. The number of HMW-GS alleles with good processing quality is limited in bread wheat cultivars, but there are plenty of HMW-GS alleles in wheat-related grasses to exploit high-molecular-weight glutenin subunits are important determinants of wheat dough quality as they give the dough visco-elastic properties required for mixing and baking performance. Because of that HMW-GS alleles are key markers in breeding programs. In this study, we describe the use of the PCR markers *Glu-1Bx7*, *Glu-1Dx2*, *Glu-1Dx5*. Using the STS-PCR method, after performing sequencing, it was possible to obtain sequences having the following lengths: *Glu-1Bx7*- 287bp, 357bp *1Dx2*- *Glu-Glu-1Dx5*-414bp. The examined sequences that were derived from different cultivars of *Triticum aestivum* L. appear to be highly homologous whereas polymorphic sites identified in them are primarily of single nucleotide polymorphisms (SNPs). These small differences in nucleotide sequences make huge differences in the quality of wheat.

## **New winter wheat DH lines with high intergeneric crossability**

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The success of wide hybridization methods aiming to transfer desirable new genetic variability from more distantly related species into cultivated wheat genotypes depends greatly upon the degree of cross compatibility (crossability) between wheat and donor species.

Researches on crossability revealed that alleles at Kr loci located on group 5 homoeologous chromosomes and on 1A are major factors controlling cross compatibility: the recessive alleles acting as promoters.

Unfortunately, the most of modern wheat genotypes carry dominant restrictive alleles at Kr loci thereby reducing the chances of their use as recipient parents. For this reason the intervarietal transfer of recessive alleles either by individual chromosome substitution or by backcrossing had been the subject of several research past programs. However, these methods are time consuming so that the recipient parent is often surpassed as regard agronomic values by other new wheat cultivars.

The aim of present study was to develop new crossable DH lines by using Zea system, with superior agronomic performances but carrying kr1k1kr2kr2 inherited from Martonvasari 9 and an allelic variant at kr1 or kr2 loci from a modern type of wheat F.132 (1-30), well adapted to local condition. Testcrosses with rye (cv. Harkovskaia) in three different years facilitated identification of some DH lines with high levels (60-70%) of intergeneric crossability.



**Marker-assisted development of bread wheat near-isogenic lines having different combinations of *Pp* (purple pericarp) alleles**

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Purple grain colour of bread and durum wheat is caused by accumulation of anthocyanins in the pericarp and is determined by the *Pp-1* and *Pp3* genes. Potential health benefits and adaptability called an interest to wheat with high anthocyanin content. The goal of the current study was to develop a set of bread wheat (*Triticum aestivum* L.) near isogenic lines (NILs) carrying different combinations of the *Pp* alleles and to demonstrate usability of these lines for dissection of 'Purple pericarp' trait. Marker-assisted backcrossing approach based on the microsatellite markers was used to develop a set of NILs with different combinations of dominant and recessive alleles at the *Pp3* (chromosome 2A), *Pp-A1* (7A) and *Pp-D1* (7D) loci. The gene *Pp-A1* is a novel locus described in wheat. qRT-PCR approach was exploited to analyze transcriptional levels of the key anthocyanin biosynthesis structural genes *Chi* (chalcone-flavanone isomerase) and *F3h* (flavanone 3-hydroxylase) in the pericarp of the NILs carrying different combinations of the *Pp* alleles. It was concluded, that the *Pp* genes up-regulated transcription of the anthocyanin biosynthesis structural genes in the pericarp, but they did it in different way at the different stages of the biosynthesis. The lines developed in the current study are a valuable model for the further comparative studies of the purple grain color effect on various characteristics of wheat plant and wheat products. The reported study was partially supported by RFBR, research project No. 14-04-31637.

**Analysis of the flanking sequences of the heterochromatic JNK region in *Secale vavilovii* Grossh. chromosomes**

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The JNK sequences are highly methylated and 1200 bp repeat unit. This motif is repeated 4000 times in the additional heterochromatin band. Flanking sequences of JNK region, which form extra heterochromatin in 2R rye chromosomes, were studied using genome walking technique. The results clearly indicated that there were blocks of JNK sequences adjacent to the R173 family of repeated sequences. Moreover, it appears that the R173 are sequences flanking in both directions, i.e. upstream and downstream. Downstream, the R173 is adjacent to the JNKs in an anti-parallel orientation, while upstream it is adjacent in a parallel orientation. In order to confirm the presence of the R173 sequence, a fluorescence in situ hybridization was carried out. Using both JNK and R173 molecular probes, overlapping hybridization signals in the 2RL pair of chromosomes were observed, indicating an identical location of the two sequence elements.

## **Cloning and characterization of the barley anthocyanin biosynthesis regulatory gene *Ant1***

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Anthocyanins are implicated in plant resistance to a number of abiotic and biotic stress factors. Anthocyanin pigmentation of barley leaf sheath is determined by the *Ant1* gene located on chromosome 7HS. The goal of the current study was sequencing of the *Ant1* gene and characterization of its dominant and recessive alleles in near-isogenic lines (NILs) of the cultivar Bowman. Three NILs having dominant *Ant1* alleles were compared with Bowman (recessive *ant1*). Microsatellite genotyping revealed donor segments in chromosomes 7H of the NILs with dominant *Ant1*, overlapping in the region between the markers *Xgbms0226* and *Xgbms0240*. The *Ant1* orthologue's (the maize gene *C1* encoding R2R3 MYB factor regulating anthocyanin biosynthesis) sequence was used for the homology-based cloning and sequencing in Bowman and its NILs. The isolated sequence was designated *HvMpc1* (*Hordeum vulgare* MYB-like protein C1). It differed in the lines with dominant and recessive *Ant1* alleles by putative functional mutations in the promoter region as well as by non-functional mutations in the coding region. The structural divergence between *HvMpc1* alleles at the promoter region may underlie their different expression: Bowman's *HvMpc1* is not transcribed, whereas it is active in leaf sheaths of the lines carrying dominant *Ant1*. Expression of the anthocyanin biosynthesis structural genes (*Chi*, *F3h*, *Dfr*, and *Ans*) was increased significantly in the presence of the dominant *Ant1* alleles. Overall, we conclude that the *Ant1* gene matches *HvMpc1* and encodes the R2R3 MYB regulatory factor. The reported study was partially supported by RFBR.

## Association Analysis of Microsatellite Markers with Morphological Traits of Grain in Bread Wheat Varieties of PBGI

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During three growing seasons (2010/11, 2011/12, 2012/13) the grain samples of 47 bread winter wheat varieties, that have been developed in PBGI in period from 2004-2012, was analyzed by extracting of 5 morphological traits from 2D digital images: area of grain image ( $A_g$ ), perimeter ( $P_g$ ), length ( $L_g$ ), width ( $W_g$ ) and grain circularity ( $C_g$ ). Allelic composition of the samples was also determined using 17 MS-markers. There was shown significant associations of alleles of MS with the parameters of investigated traits. Overall 9 marker trait associations (MTAs) for  $A_g$ , 16 for  $P_g$ , 18 for  $L_g$ , 3 for  $W_g$  and 21 for  $C_g$  were found to be stable and significant in two – three growing seasons. The MS-marker *Xgwm186-5A* was significantly associated with all analyzed traits and showed stability in three years for  $A_g$ ,  $P_g$  and  $L_g$ . Markers *Xtaglgap-1B*, *Xgwm325-6D* and *Xgwm437-7D* showed stable associations with  $P_g$ ,  $L_g$  and  $C_g$  in two - three years analyzed, while *Xgwm357-1A* was significantly associated with  $A_g$ ,  $P_g$ ,  $L_g$  and  $C_g$ . 4 alleles of MS that present in genotypes of wheat varieties of PBGI for  $A_g$ , 8 for  $P_g$ , 8 for  $L_g$ , and 1 for  $W_g$  were found to significantly associated with the increase of the values, respectively, while 5 alleles for  $A_g$ , 8 for  $P_g$ , 9 for  $L_g$ , and 2 for  $W_g$  were associated with their reduction. Stable marker-trait associations can be used in breeding programmes aiming at combining the best alleles in advanced wheat genotypes with increased grain size and/or improved grain weight.

## **120 years of winter wheat breeding in Germany – a case study**

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The breeding progress of winter wheat in Germany was investigated performing a field trial with 20 cultivars. The set contained 10 entries each, released from 1891 to 1909 and from 1991 to 2010, respectively. The material was originated from gene bank collection of IPK Gatersleben. A range of agronomic traits including plant height, flowering time, plot yield as well as yield components were considered. Moreover, the harvest index has been determined. Whereas the number of grains per ear was not influenced a small increase in thousand grain weight was observed. In addition, the modern cultivars did show a reduced plant height, an increased harvest index, an earlier flowering time and produced longer spikes.

**SSR marker-based molecular characterization and genetic diversity analysis of *Echinochloa* spp germplasm collection**

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The barnyard grass belongs to *Echinochloa* species (Poaceae), and composed 35 species. Most of species were widely known as one of the worst weeds in the world, but there are two species cultivated as both of food and animal fodder called barnyard millet as distinguished from grass. The taxonomy of the genus in its modern habitats has not been completely studied yet, but the recent advanced techniques based on DNA sequencing can be a valuable tool for the characterization and evaluation of the genetic diversity within and between species. Unfortunately, it has not been studied in detail for its molecular markers. In this study we developed SSR markers from EST database of foxtail millet (*Setaria italica* (L.) and examined the transferability of 96 *Echinochloa* species collections in the National Agrobiodiversity Center of Korea. We assessed the polymorphism of 320 EST-SSR and with alleles per locus ranged from two to eight. The averaged values of gene diversity ( $H_E$ ) and polymorphism information content (PIC) for each EST-SSR marker were 0.504 and 0.453 within populations, respectively. The 96 accessions of *Echinochloa* spp. were divided into two different groups, *Echinochloa frumentacea* and *Echinochloa* spp., and the accessions of *Echinochloa* spp. were formed three subgroups. These results also matched to agronomic traits of them. This study provides the evidence of divergence between cultivar and wild type barnyard grass by EST-SSR analysis. Thus, twenty two new EST-SSR markers were useful to various applications in genetics and genomics of *Echinochloa* species germplasm.

**HPLC and metAFLP analysis of triticale lines exposed to aluminum stress**

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Aluminum stress is one of the most frequently observed in Poland and worldwide. Toxic effect of  $Al^{3+}$  ions present in acid soils leads to the inhibition of root growth and results in decreased seed yield. Al tolerance is based mostly on the ability of organic acids to chelate  $Al^{3+}$  ions via formation of low molecular weight complexes. In triticale major gene coding for aluminum-activated malate transporter (ALMT), is located on 7R chromosome and explains up to 36% of phenotypic variance. Additional loci were mapped to the chromosomes 3R, 4R and 6R. Their functions in triticale are purely unknown, however in rye the gene located on 3R codes putative STOP1 transcriptional factor that may have regulatory activity. Despite numerous evidences that the trait has genetic background, assuming that stresses could be reflected at the DNA methylation level, one may speculate that at least part of the phenotypic variance is due to epigenetic effects. Unfortunately, such studies were not carried yet. However, studies at the DNA methylation level could be easily performed via HPLC-RP and/or metAFLP approaches. The two methods were successfully used by us to study changes induced in tissue cultures. The aim of the study was to test if aluminum tolerance in triticale could be co-regulated by epigenetic processes identified at the DNA methylation level.

The experiment was based on five Al-tolerant and five non-tolerant lines that were grown under stressful (15, 20 and 30ppm of  $Al^{3+}$ ) as well as control conditions (untreated). DNA for HPLC-RP and metAFLP analysis was isolated from 0.3-0.5 cm root tips. Total genomic DNA methylation (HPLC-RP) of plants grown under control conditions equaled from 22.2% for tolerant to 22.6% for non-tolerant lines, respectively. Analysis of variance (AMOVA) and Tukey's test demonstrated increasing level (about 1%) of Cm under stressful conditions for non-tolerant lines regardless of  $Al^{3+}$  concentration ( $F=96.24$ ,  $p=0$ ;  $F=305.4$ ,  $p>0$ ;  $p=1.17e-07$ ,  $F=23.58$ ,  $p>0.001$  for 15, 20 and 30ppm of  $Al^{3+}$ , respectively). Contrary, DNA methylation of tolerant lines grown in the presence of 20 and 30ppm of  $Al^{3+}$  increased about 0.6% ( $F=7.89$ ,  $p>0.05$ ;  $F=22.8$ ,  $p>0.001$ , respectively). No statistical differences between lines grown under stressful and control conditions were noticed in the presence of 15ppm  $Al^{3+}$ . Similar analysis of the DNA samples of tolerant and non-tolerant lines performed with metAFLP approach failed to distinguish between analyzed materials at least in the case of DNA methylation.

Our data suggests that Al treatment may influence global DNA methylation of tolerant and non-tolerant triticale lines. However, such changes were not detected via metAFLP approach suggesting that some genomic regions changing their methylation status under stressful conditions may not be available for the metAFLP approach.

## Research on improvement of resistance to powdery mildew in oat

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Powdery mildew caused by *Blumeria graminis* DC. f. sp. *avenae* Em. Marchal. is one of the most important foliar disease of common oat. The annual crop losses from mildew infections range from 5-10% up to 40%. Till date 8 resistant gene to powdery mildew has been identified (*Pm1-Pm8*).

Based on host-pathogen tests resistant genes to powdery mildew in Polish oat cultivars were identified. Among 30 tested cultivars only four possessed already known resistance genes. Cultivar Dragon possess *Pm6* gene, Skrzat - *Pm1*, Deresz and Hetman - *Pm3*. Effectiveness analysis of resistant genes showed that these genes characterised low level of resistance. The most effective in Polish conditions are *Pm4* and *Pm7*, but this genes are not used in breeding programmes yet.

Large genetic changeability and ability to generate new forms by mutations and DNA recombinations makes powdery mildew easily adaptable to new conditions. Therefore there is a need to identify new and effective sources of resistance. In genus *Avena* exist many wild species which could be used as a potential sources of resistance. Our investigation showed that tetraploid species characterised high level of resistance, but because of strong sterility barriers the transfer of resistant genes cannot be achieved by means of a similar backcrossing programme. More effective transfer could be achieved using species which share all genomes with *A. sativa*. Our experiments showed that hexaploid species *A. sterilis* is also good potential donors of resistant genes.

In future work we plan characterization of new sources of resistance to powdery mildew in *A. sterilis* genotypes and transfer these genes into cultivated oat.



## Putative markers towards *Dw6* dwarfing gene in oats

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New forms characterized by high quality and increased yield of seeds as well as tolerant to biotic and abiotic stresses are the principal purpose of cereal breeding programs. Cultivation of intensive forms that positively react towards plant protection and increased fertilization enables achieving high yield. However, in the case of lodging efficient plant vegetation is diminished. Moreover, lodging exhibits detrimental effects on seed and straw quality. Lodging could be prevented via application of retardants. However, due to lack of sufficient studies and distinct reaction of oat varieties, retardants are usually not recommended. The alternative way that could be considered for lodging prevention is the use of varieties with increased tolerance supported by dwarfness genes.

In Polish breeding programs of oats, dominant dwarfness *Dw6* gene is used to shorten the straw. The forms carrying that gene are usually about 60% shorter, however, without loss of yield.

The primary aim of the study was the identification of putative *Dw6* gene markers by means of RAPD and BSA approach. The F<sub>2</sub> biparental oat mapping population ('Celer' x STH 9210) was used. The STH 9210 is a line with *Dw6* gene that has a short straw whereas 'Celer' is the Polish breeding cultivar growing up to 120 cm high.

RAPD reactions were performed on bulks combined with the DNAs of short and high F<sub>2</sub> plants. About 600 RAPD primers were tested. Only G12 primer amplified product that was present in STH 9210 and the bulk of short F<sub>2</sub> but not in the remaining samples.

## **The genetic mapping of QTLs associated with activity of different lipoxygenase forms in genome D of *Triticum aestivum* under drought**

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Using *Triticum aestivum*–*Aegilops tauschii* introgression lines, seven quantitative trait loci (QTL) (LOD threshold from 1.99 to 2.93) associated with the lipoxygenase (LOX) activity in different organs of wheat plants grown under drought were found. Activity of the soluble LOX of seed has been mapped to the short arm of chromosome 4D. Two QTL associated with the phenotypic expression of LOX activity in wheat seedlings were identified on chromosome 5D. The first is related to the membrane-bonded enzyme activity; it is located on the short arm of chromosome 5D. The second QTL associated with the activity of soluble LOX was located on the long arm of chromosome 5D. Two loci responsible for the activity of soluble LOX in wheat leaves were found on the short arm of chromosome 2D. The activity of chloroplast enzyme form was detected on 2DS, too, and was linked to the marker *Xgwm261*. On chromosome 7D both soluble and membrane-bound LOX activity were localized in the centromeric region.

The identified QTL associated with LOX activity, co-localized with the parameters of gas exchange, rate of photosynthesis, photosynthetic pigments, chlorophyll fluorescence and grain productivity in wheat under drought. Probably, the different forms of LOX are differentially involved in the adaptation of wheat to water deficit.

**Association of introgressions in 2A, 2B and 5A chromosomes of bread wheat from *Triticum timopheevii* Tausch. with parameters of gas exchange, chlorophyll fluorescence and activity of antioxidant enzymes under normal and water deficit conditions**

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Alien hybridization in cereals is used for comparative investigations of genome structure and evolution as well as for extracting the useful genes from a wild gene pool. The tetraploid species *Triticum timopheevii* has long been used as a source of genes for resistance to fungal diseases. The line 821 was developed by E. Budashkina on the genetic background of drought resistant but very susceptible for diseases cultivar Saratovskaya 29 (S29), and resistant to leaf rust. According the genotyping data, the line carries big introgressions in 2A and 2B chromosomes and a small introgression in subtelomeric region of 5A chromosome. The line was studied for a number of physiological and biochemical parameters under conditions of normal and restricted water supply. It was found that the line substantially differed from the initial drought tolerant cultivar for gas exchange, chlorophyll fluorescence and activity of antioxidant enzymes under both conditions. Under normal watering the parental cultivar differed by decreased parameters of gas exchange and photosynthesis comparing to the line 821 but had higher water use efficiency (WUE). Under drought, the line noticeably lowered these parameters in contrast to S29 in which these parameters increased and WUE was also substantially higher. In addition, the activity of antioxidant enzymes, dehydroascorbate reductase and catalase, inactivating an excess of reactive oxygen species considerably elevated and WUE was higher. Thus, it may be supposed that 2A, 2B and 5A chromosomes carry the important genetic complexes responsible for reaction of living systems of wheat plant on changeable environment.

## **Genomics of alien gene transfer in wheat: towards the understanding of interaction between a host genome and introgressed chromosomes**

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Since more than a century, wheat has been crossed with its wild relatives in attempt to introduce favourable alleles, genes and gene complexes providing resistance to biotic and abiotic stresses and other beneficial traits. Although hybridization with wild species represents a powerful approach for crop improvement, the knowledge of underlying biological mechanisms remains limited and only a few wheat-alien introgression lines have made their way into the agriculture. This project aims to provide novel information on the interaction between a host genome and introgressed chromosomes and their parts, both at genome and transcriptome levels. We have chosen wheat-barley ditelosomic addition line 7HL, which was obtained after hybridization between bread wheat cv. Chinese Spring and barley cv. Betzes, as a model system. The addition line 7HL contains a complete set of wheat chromosomes and a pair of long arms of barley chromosome 7H, and exhibits interesting agronomic traits such as salt tolerance, earliness and increased  $\beta$ -glucan content in the grain. We have sequenced transcriptomes of both parents and the ditelosomic addition line 7HL to reveal differences in gene expression due to the intergenomic interactions. As a first step, we compared expression of 7HL genes in the natural background (in barley parent) with the expression of the same gene set in the wheat host background. While a majority of 7HL genes were down-regulated in the host background, some genes were up-regulated. This analysis provides new data to understand the regulation of alien genes in a recipient genome.

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**Isolation and functional characterization of the *Pp3* gene, required for purple caryopsis pericarp trait formation**

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Functional food rich in anthocyanin content has important role in human diets, reducing diseases risk. Common wheat with purple colored grains is considered as a source of these healthy molecules, which accumulated in caryopsis pericarp. Two complementary *Pp* genes required for activation of anthocyanin biosynthesis (AB) in wheat pericarp: one of the *Pp-1* homoealleles (mapping to each of the group 7 chromosomes) and *Pp3* (on chromosome 2A). In the current study, a candidate gene for *Pp3* was isolated and characterized for the first time. This gene, *TaMyc1*, encodes MYC-like regulatory factor harboring a conserved basic helix-loop-helix (bHLH) domain and activating transcription of the anthocyanin biosynthesis structural genes. In genotypes characterized by dominant *Pp3* allele, *TaMyc1* is actively expressed in pericarp and, at the lower levels, in coleoptile, culm and leaves. *TaMyc1* is located on chromosome 2A, where the *Pp3* locus has been previously mapped, and has at least three additional copies (one per homologous chromosomes of group 2) not expressing in pericarp. Quantitative assay of the *TaMyc1* mRNA level in the presence of different combinations of the recessive and dominant *Pp3* and *Pp-1* alleles demonstrated partial suppression of the *TaMyc1* transcription by the dominant *Pp-D1* allele. The reported study was partially supported by RFBR, research project No. 14-04-31637.

## **The contribution of the alien translocations into *Chi* total expression in wheat introgression lines**

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Alien genetic material is widely used now for improvement of bread wheat (*Triticum aestivum* L.,  $2n = 6x = 42$ , BBAADD). The species *T. timopheevii* ( $2n = 4x = 28$ , GGAA) and *Aegilops speltoides* ( $2n = 2x = 14$ , SS) can be donors of alleles for specific resistance to various phytopathogenes and a potential source of genes conferring tolerance to abiotic stress factors. The *Chi* gene belongs to a large group of the plant defense genes. *Chi* encodes chalcone-flavanone isomerase (CHI; EC 5.5.1.6.) – enzyme catalyzing cyclization of chalcone into (2S)-naringenin participates in synthesis of nine major classes of flavonoid compounds. In the current study, the genes *TaChi1* of *T. aestivum*, *AsChi1* of *Ae. speltoides*, and *TtChi1* of *T. timopheevii* were isolated, characterized and mapped using wheat-alien single introgression lines (Saratovskaya 29-*T. timopheevii* 5B/5G and Rodina-*Ae. speltoides* 5B/5S) as well as bread wheat nullitetrasonic and deletion lines. The genes *TaChi1*, *AsChi1* and *TtChi1* are orthologs localized in the long arm of Triticeae chromosome 5. Three *Chi* homoeologous copies in bread wheat (*TaChi-A1*, *-B1*, *-D1*) essentially diverged in the regulatory regions and demonstrate different transcriptional levels either in control conditions or in response to salinity. Replacement of *TaChi-B1* by *AsChi1* or *TtChi1* resulted in tissue-specific changes of the total *Chi* expression in wheat-alien introgression lines: decrease of *Chi* transcript in coleoptiles, but maintaining it at the same level in roots. The reported study was partially supported by RFBR, research project No. 14-04-31637.

**Application of ISSR-PCR, IRAP-PCR, REMAP-PCR and ITAP-PCR in the assessment of genomic changes in the early generation of triticale**

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Analysis of structural changes of the octoploid triticale genomes was conducted in F<sub>2</sub> and F<sub>3</sub> generations. The plants were derived from crosses of five cultivars and breeding lines of hexaploid wheat (*Triticum aestivum* L.) with a variety of cultivated rye (*Secale cereale* L.). The study used four marker systems: ISSR (inter-simple sequence repeat), IRAP (inter-retrotransposon amplified polymorphism), REMAP (retrotransposon-microsatellite amplified polymorphism) and the technique developed by the authors named ITAP (inter-transposon amplified polymorphism).

Most frequently, elimination of specific bands was observed, especially of rye bands. Depending on the cross combination, the percentage of eliminated rye bands ranged from 73.6% to 80.6%. The lower percentage of wheat bands was eliminated, i.e., from 57.6% to 76.48%, depending on the combination of crosses. The emergence of new types of bands in hybrids, absent in the parental forms was the rarest phenomenon (14.5-17.9%). The results indicate the ongoing process of genome rearrangements at the molecular level in the early generations of plant crosses that also involve repeated nucleotide sequences of DNA.

## Phenotyping approaches to the genes affecting flowering time

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Special genotypes of wheat have been produced with the aim of searching for better adapted combinations of genes and alleles influencing yield. These mainly include genes and alleles controlling flowering time and vernalization responses.

Two cultivars, Paragon and Kaerntner Frueher, have 11-day difference in heading time, but no difference in main flowering time genes (*Ppd-D1*, *Vrn-A1*, *Vrn-B1*, and *vrn-D1*). To specify the source of this interesting contrast, both cultivars were crossed and the mapping population was developed.

Recently described and widely distributed novel allele *Vrn-B1c* among the East European and Russian cultivars of spring wheat was the source for present study of the effect on the vernalization requirements. Using cultivars Granny and Trappe as donors of dominant alleles *Vrn-B1c* and *Vrn-B1a*, respectively, development of NILs by backcrossing has been done.

The research leading to these results has received funding from the European Union Seventh Framework Programme ADAPTAWHEAT under grant agreement n° 289842 (FP7-KBBE-2011-5) and from Ministry of Agriculture project no. MZe RO-0414.



## **Genotyping and linkage analysis of wheat experimental populations using KASP platform**

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The objective of this paper was to validate usefulness of kompetitive allele specific polymerase chain reaction (KASP) assays for evaluation of genetic variation and for construction of linkage maps in wheat. For this purpose, two sets of KASP markers belonging to the core set of 960 KASP assays available from CerealsDB, were used for molecular evaluation of two F7 SSD mapping populations, NS36-98/Paragon and Magnif41/Paragon, consisted of 83 and 112 lines, respectively. These populations were developed in collaboration between IFVC and JIC to identify QTL controlling canopy architecture and studied as part of the EU FP7 project ADAPTAWHEAT. Linkage maps were constructed using MapDisto 1.7.6.5 software and Kosambi mapping function.

A total of 154 KASP markers, polymorphic between parents were applied for evaluation of NS36-98/Paragon population. 143 markers were distributed in 30 linkage groups, while 11 markers were unassigned. Total map size was 947 cM. Genetic variation of the second population was accessed by 75 markers common with the first set and with additional 54 markers polymorphic between Magnif41 and Paragon. Total map size was 960 cM, with 118 markers assigned in 28 linkage groups, while 11 markers remained unassigned. According to obtained results, KASP markers are a good choice for further QTL analysis and marker assisted selection in these wheat populations. The common parent Paragon, is shared in a number of populations within the UKs Wheat Improvement Strategic Programme (WISP) and taken together these linked materials can be used to conduct a joint analysis of key traits.

This research was partly funded by the European Union via FP7 project ADAPTAWHEAT (project number: 289842) and partly by the MSTD of the Republic of Serbia (project number TR-31066).

## **The effect of alien introgressions in wheat genome on drought and salinity tolerance**

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Drought and salinity are among the major constraints that affect plant growth and yield. To improve complex stability of bread wheat (*Triticum aestivum* L.) development of new wheat genotypes carrying introgressions from other cereal species is widely applied. The aim of this study was estimation of the foreign genetic material (derived from *Aegilops speltoides* and *T. timopheevii*) effect on drought and salinity stress tolerance of wheat seedlings. Indirect evaluation of drought resistance by creating an artificial shortage of moisture in the laboratory conditions (using 15% PEG solution) has identified positive influence of translocation T6BS • 6BL-6SL from *Ae. speltoides* and negative effect of *T. timopheevii* introgression in chromosome 2A. On the example of translocation T5BS • 5BL-5SL, it was shown that the same foreign fragment introgressed into different wheat genotypes can have different effects on resistance to osmotic stress depending on the drought tolerance degree of the initial wheat genotype. Evaluation of salinity tolerance in the laboratory conditions by using 150 mM NaCl solution has identified positive influence of translocation T5BS • 5BL-5SL and *T. timopheevii* introgression in chromosome 2A, while the presence of *T. timopheevii* introgression in chromosome 5B and translocation T6BS • 6BL-6SL, on the contrary, reduced the resistance of wheat to salt stress. The reported study was partially supported by RFBR.

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