III National Conference

GENETICS AND GENOMICS IN IMPROVING PLANTS – FROM MODEL PLANT TO NEW VARIETY

5-7 November 2014
Poznań

Program and abstracts

INSTITUTE OF PLANT GENETICS
POLISH ACADEMY OF SCIENCES
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Dear IPG PAS Conference Attendees,

The advances in research on plant genomes and molecular systsems have led to a dynamic progress in the areas of biological and agricultural sciences that can benefit from better knowledge on the plant functions. The modern plant breeding also uses techniques that allow for a knowledge-based usage of genetic materials. Polish membership in European Union stimulates further research, but also opens new possibilities of practical applications of scientific results. We hope that during the Conference you will have an opportunity to present your achievements in all fascinating areas of plant science that you represent and discuss them with other researchers and breeders.

Prof. dr hab. Bogdan Wolko
Director of the Institute of Plant Genetics PAS

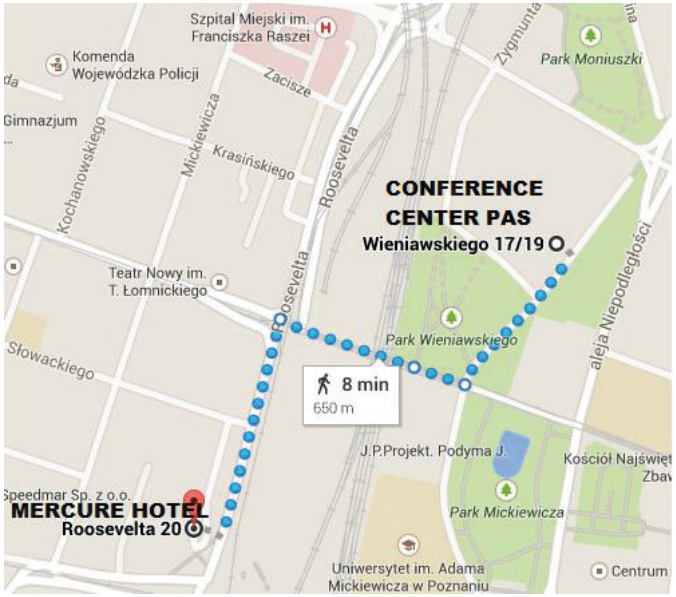
CONFERENCE VENUE

III National Conference IPG PAS will be held at:

Conference Center,
Polish Academy of Sciences,
Wieniawskiego 17/19, 61-713 Poznań, Poland

Dinner party will be held at:

Hotel Mercure,
Roosevelta 20, 60-829 Poznań, Poland



PRESENTATION INSTRUCTION

Oral Presentations

Each contributed oral presentation will be allocated 20 minutes for presentation and questions. Please ensure that you plan your presentation to fit this schedule.

Poster Presentations

Posters can be displayed from 8:00 on Thursday, 6-th of November 2014, and can stay on display until the end of conference. Please assign your poster to a poster board according to the number listed in the program.

IPG PAS Conference program

5 November 2014	6 November 2014	7 November 2014
13:00 – 15:00 Registration	9:00 – 9:45 Invited lecture: Claudia Jonak	9:00 – 9:45 Invited lecture: Ernesto Igartua
	9:45 – 11:05 Session 2: Stress response mechanisms	9:45 – 11:05 Drought tolerance - POLAPGEN-BD project open workshop - part II
	11:05 – 11:30 Coffee break	11:05 – 11:30 Coffee break
	11:30 – 12:15 Invited lecture: Kerstin Kaufmann	11:30 – 14:10 Drought tolerance - POLAPGEN-BD project open workshop - part III
	12:15 – 13:15 Session 3: Plant-microorganism interactions	14:10 – 14:15 Conference closing
	13:15 – 14:15 Lunch	14:15 – 15:00 Lunch
15:00 – 15:05 Opening	14:15 – 15:00 Invited lecture: Jan Szopa-Skórkowski	15:00 – 18:30 Session: POLAPGEN-BD project session (internal matters)
15:05 – 15:50 Inauguration lecture: Robert Hasterok	15:00 – 16:00 Session 4: Biotechnology in basic and applied research - part I	
15:50 – 16:30 Session 1: Genome structure, function and evolution	16:00 – 16:30 Coffee break	
16:30 – 17:00 Coffee break	16:30 – 17:50 Session 4: Biotechnology in basic and applied research - part II	
17:00 – 19:20 Drought tolerance - POLAPGEN-BD project open workshop - part I	19:30 – 2:00 Dinner party	

PROGRAM

WEDNESDAY, 5 NOVEMBER 2014

13:00 – 15:00 Registration

15:00 – 15:05 Opening

15:05 – 15:50 Inauguration lecture

Robert Hasterok (Uniwersytet Śląski): *Brachypodium - a model genus to study grass genome organisation at the cytomolecular level*

Session 1: Genome structure, function and evolution – Chair: Wojciech Święcicki

15:50 – 16:10 Michał Książkiewicz**:** *Tracking legume ancestral genome: insights from the comparative mapping of L. angustifolius gene-rich regions*

16:10 – 16:30 Michał Kwiatek: *Development of the genetic diversity of triticale (× Triticosecale Wittm.) by the intergeneric crosses with goatgrasses (Aegilops spp.)*

16:30 – 17:00 Coffee break

Drought tolerance - POLAPGEN-BD project open workshop - part I – Chair: Maciej Stobiecki

17:00 – 17:20 Andrzej Kędziora: *Present and future agrometeorological conditions of the crop of spring barley in Poland*

17:20 – 17:40 Damian Wach: *Tolerance of spring barley lines to temporal drought stress in relation to grain yield and yield components*

17:40 – 18:00 Krzysztof Mikołajczak: *Mapping of QTLs for the plant height and yield forming traits in RIL population of spring barley (Hordeum vulgare L.) under various environments*

18:00 – 18:20 Małgorzata Łukowska: *Contact angle and surface free energy of plant leaves and their changes under drought conditions.*

18:20 – 18:40 Filip Mituła: *Hordeum vulgare calcium-dependent protein kinase 34 regulates drought stress response resent and future agrometeorological conditions of the crop of spring barley in Polan*

18:40 – 19:00 Justyna Niedziela: *Drought-induced free proline synthesis and ABA accumulation in leaves and roots of spring barley genotypes of different origin*

19:00 – 19:20 Barbara Swarcewicz: *Changes in metabolite profiles of barley (Hordeum vulgare L.) subjected to drought stress*

THURSDAY, 6 NOVEMBER 2014

9:00 – 9:45 Invited lecture

Claudia Jonak (Gregor Mendel Institute of Molecular Plant Biology, Austria) *Phosphorylation-mediated stress signalling and redox regulation*

Session 2: Stress response mechanisms – Chair: Jan Sadowski

9:45 – 10:05 Grzegorz Józefaciuk**:** *Unknown mechanism of plant reaction to drought: changes in surface charge and acidity of roots*

10:05 – 10:25 Aleksandra Świda-Barteczka**:** *Platform for analysis of barley pri-miRNA expression reveals a new mechanism of drought induced regulation of miRNA 444.3a level*

10:25 – 10:45 Andrzej Pacak**:** *Heat stress responsive microRNA inhibit tillering in barley*

10:45 – 11:05 Agata Cieśla**:** *Arabidopsis protein phosphatase 2C ABI1 regulates type III ACS protein turnover*

11:05 – 11:30 Coffee break

11:30 – 12:15 Invited lecture

Kerstin Kaufmann (University of Potsdam, Germany) *Dynamics and evolution of gene regulation by MADS-domain transcription factors in Arabidopsis flower development*

Session 3: Plant-microorganism interactions – Chair: Cezary Mądrzak

12:15 – 12:35 Joanna Kaczmarek**:** *Identification, quantification and characterization of selected oilseed rape pathogens in air samples*

12:35 – 12:55 Paweł Serbiak**:** *Fusarium community on wheat grain samples originating from different regions of Poland in 2013*

12:55 – 13:15 Małgorzata Jędryczka**:** *Sources of genetic resistance to Plasmodiophora brassicae*

13:15 - 14:15 Lunch

14:15 - 15:00 Invited lecture

Jan Szopa Skórkowski (Uniwersytet Wrocławski) *EMO, the alternative for GMO*

Session 4: Biotechnology in basic and applied research - part I – Chair: Zofia Banaszak

15:00 – 15:20 Andrzej Wojciechowski**:** *The use of in vitro embryo rescue cultures for introduction of resistance genes from related Brassica species into oilseed rape (Brassica napus L.)*

15:20 – 15:40 Katarzyna Sosnowska**:** *Extending the oilseed rape gene pool with resynthesis Brassica napus L.*

15:40 – 16:00 Anna Trojak-Goluch**:** *Development of tobacco doubled haploids resistant to tomato spotted wilt virus and Thielaviopsis basicola using biological assays and SCAR markers*

16:00 – 16:30 Coffee break

Session 4: Biotechnology in basic and applied research - part II – Chair: Zofia Banaszak

16:30 – 16:50 Marcin Czyż**:** *Freeze-drying of plant tissue containing HBV surface antigen for the oral vaccine against hepatitis B*

16:50 – 17:10 Monika Langner **:** *Characterisation of HMW glutenin subunits in common wheat by cIEF*

17:10 – 17:30 Maja Boczkowska**:** *Tissue-specific expression analysis of cytokinin dehydrogenase genes in common wheat*

19:30 – 2:00 Dinner party – Hotel Mercure

FRIDAY, 7 NOVEMBER 2014

9:00 – 9:45 Invited lecture

Ernesto Igartua (Estación Experimental de Aula Dei CSIC, Spain) *Adaptation in barley, with emphasis on photoperiod and vernalization responses*

Drought tolerance - POLAPGEN-BD project open workshop - part II – Chair: Paweł Krajewski

9:45 – 10:05 Justyna Guzy-Wróbelska: *A high density 'function map' aimed at the dissection of drought tolerance related QTL in barley*

10:05 – 10:25 Piotr Ogrodowicz: *Identification of QTL associated with yield and earliness in barley RIL lines derived from hybrids between European and Syrian varieties differentiated in tolerance to water deficiency*

10:25 – 10:45 Agata Daszkowska-Golec: *The functional analysis of candidate genes related to drought response in barley using TILLING strategy*

10:45 – 11:05 Katarzyna Kruszka: *The role of micro RNA in regulation of mechanisms leading to drought adaptation*

11:05 – 11:30 Coffee break

Drought tolerance - POLAPGEN-BD project open workshop - part III – Chair: Zofia Szweykowska-Kulińska

11:30 – 11:50 Małgorzata Kaczmarek: *Analysis of calcium dependent protein kinase (CDPK) genes expression during drought adaptation in barley (Hordeum vulgare L.)*

11:50 – 12:10 Agnieszka Janiak: *Differential analysis of barley leaves and root transcriptomes under drought stress and its application for molecular markers development*

12:10 – 12:30 Paweł Rodziewicz: *Proteomic analysis of barley mapping population subjected to drought*

12:30 – 12:50 Maria Filek: *Characteristics of the diversity of parental and reference lines of barley in terms of changes of the content of proline, sugars and ethylene under the drought stress*

12:50 – 13:10 Anna Piasecka: *Phenolic metabolites expression in leaves of barley inbred lines - comparison of greenhouse and field experiment*

13:10 – 13:30 Michał Dziurka: *Physiological response to water stress at seedling stage of spring barley lines*

13:30 – 13:50 Tadeusz Rorat: *Barley genotypes display differential physiological and molecular response to progressive water deficit*

13:50 – 14:10 Tomasz Wyka: *Structural modifications induced by a drought/rewatering cycle in barley leaves*

14:10 – 14:15 Conference closing

14:15 – 15:00 Lunch

15:00 – 18:30 POLAPGEN-BD project session (internal matters)

Inauguration lecture

Brachypodium – a model genus to study grass genome organisation at the cytomolecular level

Robert Hasterok, Alexander Betekhtin, Natalia Borowska, Agnieszka Braszewska-Zalewska, Ewa Breda, Karolina Chwiałkowska, Renata Górkiewicz, Dominika Idziak, Jolanta Kwaśniewska, Mirosław Kwaśniewski, Dorota Siwińska, Anna Wiszyńska, Elżbieta Wolny

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In contrast to animals, the organisation of plant genomes at the cytomolecular level is still relatively poorly studied and understood. However, the *Brachypodium* genus in general and *B. distachyon* in particular represent exceptionally good model systems for such study. This is due not only to their highly desirable 'model' biological features, such as small nuclear genome, low chromosome number and complex phylogenetic relations, but also to the rapidly and continuously growing repertoire of experimental tools, such as large collections of accessions, WGS information, large insert (BAC) libraries of genomic DNA, etc. Advanced cytomolecular techniques, such as fluorescence *in situ* hybridisation (FISH) with evermore sophisticated probes, empowered by cutting-edge microscope and digital image acquisition and processing systems, offer unprecedented insight into chromatin organisation at various phases of the cell cycle. A good example is chromosome painting which uses pools of chromosome-specific BAC clones, and enables the tracking of individual chromosomes not only during cell division but also during interphase.
This presentation outlines the present status of molecular cytogenetic analyses of plant genome structure, dynamics and evolution using *B. distachyon* and some of its relatives. The current projects focus on important scientific questions, such as: What mechanisms shape the karyotypes? Is the distribution of individual chromosomes within an interphase nucleus determined? Are there hot spots of structural rearrangement in *Brachypodium* chromosomes? Which epigenetic processes play a crucial role in *B. distachyon* embryo development and selective silencing of rRNA genes in *Brachypodium* allopolyploids?

The authors acknowledge financial support from the Polish National Science Centre (grants no. 2012/04/A/NZ3/00572 and 2011/01/B/NZ3/00177)

Invited lectures

Invited lecture

Phosphorylation-mediated stress signalling and redox regulation

Claudia Jonak

GMI - Gregor Mendel Institute of Molecular Plant Biology, Dr. Bohr-Gasse 3, 1030 Vienna, Austria

High soil salinity is a major environmental constraint for plant growth and development and a worldwide phenomenon that negatively affects agricultural productivity. Plants respond to salinity stress with an array of mechanisms including transcriptional and metabolic rearrangements, reduced cell division and expansion, and enhanced senescence. These responses are delicately coordinated by signalling pathways that ultimately result in tolerance or sensitivity. Protein kinases constitute important regulators in these circuits. In this seminar I will focus on mechanisms of stress signal transduction and metabolic adjustments.

Invited lecture

Dynamics and evolution of gene regulation by MADS-domain transcription factors in Arabidopsis flower development

Alice Pajoro[1], Jose Muino[2], Pedro Madrigal[3], Cezary Smaczniak[1], Suzanne de Bruijn[1,8], Paweł Krajewski[4], Jose-Luis Riechmann[5,6], Gerco Angenent[1,7], and **Kerstin Kaufmann**[1,8]*

[1]*Laboratory of Molecular Biology, Wageningen University, Wageningen , The Netherlands*
[2]*Max-Planck Institute for Molecular Genetics, Department of Computational Molecular Biology, Berlin, Germany*
[3]*Wellcome Trust Sanger Institute, Hinxton, UK*
[4]*Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland*
[5]*Center for Research in Agricultural Genomics-CSIC-IRTA-UAB-UB, Barcelona,Spain*
[6]*Institució Catalana de Recerca i Estudis Avançats-ICREA, Barcelona, Spain*
[7]*Business Unit Bioscience, Plant Research International, Wageningen, The Netherlands*
[8]*Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany*

MADS-domain transcription factors act as master regulators of developmental switches and organ specification in plants. However, the molecular mechanisms by which these factors dynamically and specifically regulate the expression of their target genes during development are still poorly understood. We characterized changes in chromatin accessibility, gene expression and DNA-binding of two MADS-domain transcription factors (TFs) during Arabidopsis flower development. Our findings suggest that the MADS-domain factors APETALA1 and SEPALLATA3 act as 'pioneer factors' that modulate chromatin accessibility and thereby control gene expression and enhancer activity. In order to study how different MADS-domain TFs achieve their functional specificity, we used a SELEX-seq approach to reveal DNA-binding specificity of different MADS domain protein complexes that specify distinct organ types in the flower. Our findings support the idea that functional specificity of MADS-domain proteins is at least partly established by differences in DNA-binding.
A second major research topic in my group is to understand the evolution of gene regulation by floral MADS domain factors. Based on mutant phenotypes and expression patterns, the functions of MADS domain proteins appear to be conserved across flowering plants, despite large morphological variation in flower development. Using a comparative ChIP-seq approach, we have determined the evolutionary variation of DNA binding sites of a flower developmental key regulatory MADS TF in two closely related species: *Arabidopsis thaliana* and *A. lyrata*. Despite their recent evolutionary divergence of ~10 Mio years, we found a high level of divergence between TF binding site locations. Only ~25 % of the sites are shared between the two species. Conservation of binding sites is influenced by their genomic context, such as distance to the TSS. Binding sites that are at larger distances from nearby genes are maintained largely independently of shifts in their position. In *A. lyrata*, we found that binding sites can be associated with transposable elements, which likely contributed to the origin of novel binding site locations in this species.

EMO, the alternative for GMO

Jan Szopa-Skórkowski

Wrocław University

The development of nucleic acid sequencing methods resulted in the stream of data on genes structure. The progress on genes structure forced the development of research on identification of their function. For evaluation of gene function, the most effective method is the generation of an organism with overexpression or/and repression or/and complementation of repression/overexpression of the gene with the subsequent analysis of modified organism. GM (genetically modified) plants, generated for over three decades, provided the valuable information of gene function, and simultaneously indicated the advantages of transgenic plants.
According to the European Commission, the challenge for biotechnology, as a scientific discipline, is searching for tools for agriculture and industry development and providing such a plant productivity, that will satisfy growing food demand. To rise this challenge, the generation and application of GM plants is justified.
At the turn of 2010/2011 the European Commission released a study summing up the ten-year (2001-2010) research on GM plants (A decade of EU-funded GMO research). The main conclusion summarizing these research is, that the cultivation, processing and using of GM plants is not more risky than those of conventional cultivation. Even though, another study released by the European Commission (Europeans and Biotechnology in 2010) says, that over 60% (61-90% depending of the country) of the society does not accept GMO.
From two decades we (*Linum* Foundation, www.linum.pl) have been generating GM potatoes and nowadays GM flax. At first, the aim was to identify the genes function and their significance for plant productivity and metabolism. The most attention was given to those genes, that are putatively key genes for plant infection resistance, are crucial for regulation of the synthesis of the compounds of biomedical functions (phenolic acids, flavonoids, terpenes) or are applicable in industry (fatty acids) and in the environment protection (biodegradable polymers). We generated plants of elevated resistance to pathogen infection, of increased accumulation of phenylpropanoids and terpenoids, of altered synthesis and accumulation of biopolymers (pectins, lignins, cellulose, hemicellulose) and biodegradable polymers (polyhydroxybutyrate) and flax of altered fatty acids profile. We estimated the influence of GM flax cultivation on soil ecosystem and we revealed the intensified and beneficial interaction of mycorrhized organisms on flax root system. We showed the positive influence of GM flax seeds consumption on the broad range of parameters describing health condition of laboratory animals (mice, rats). We generated a few preparations applicable in the prevention and treatment of people, including certified flax dressing LENPLAST for chronic wounds treatment, certified preparations of anti-inflammatory and antibacterial function (LINFIX) and of skin regenerating function (OILFIX), diet supplement LINACTIVE beneficial in anti-inflammatory and anticancer prevention, admitted to the trade by GIS and we offer flax oil of the characteristics of ideal oil and other preparations of antibacterial function.
The favourable characteristics of GM flax and the preparation generated from GM flax cannot be produced on the large- scale due to the difficulties in obtaining agreement on GM plants release into the environment for production. Although unjustified but still strong social reluctance to GMO and the restrictive regulations forced the elaboration of new technology using the knowledge resulting from GM plants analysis and using it to the generation of favourable altered plants omitting the introduction to their genome the heterologous genes. Two-year experiments led to the elaboration of EMO (epigenetically modified organism) technology resulting in the profitable changes in plants without their modification by the vector transgenesis. Briefly, the first stage of the method comprises the induction of the changes in the endogenous gene by its methylation/demethylation or the changes in accumulation of gene derived products (mRNA). In the second stage, the plants are selected by molecular markers derived from the analysis of GM plants. In the third stage, the establishment of introduced changes by the exposition of plants to the factors increasing DNA methylation level (mannitol, salicylic acid, jasmonates) or mobilizing the activity of the whole genome (infection by non-virulent microorganisms) takes place. The comparative analysis of flax, in which the lycopene cyclase gene was induced by a vector method (GMO) and non-vector method (EMO), revealed the two time higher effectiveness of the latter. Beside from the evidently higher effectiveness, the most important is, that the EMO method allow to generate the favorably altered plants, whose cultivation make the plant producer independent form the complicated and unstable procedure of obtaining agreement on their release into environment.

Discoveries in barley landraces. No need to be lucky anymore.

Ernesto Igartua, Ana M. Casas

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Crop landraces or traditional varieties are local breeds of crops that have evolved in the same place over a long time, accumulating adaptations to both the constraints posed by the environment and the requirements of producers and consumers. In developed countries, cereal landraces are now mostly kept in germplasm banks for two reasons: in general, they have been surpassed in agronomic performance by new cultivars, and trait and gene mining in landraces was hampered by linkage drag. These resources have been at the disposal of plant breeders for a long time and, indeed, they have been used extensively, although every progress achieved implied great efforts and a bit of luck.

The genomics revolution has opened new opportunities for gene and allele mining in landraces and crop wild relatives, and their effective introduction in breeding programs. The challenges to make full use of this information are now more on the quantity and quality of the information contributed by phenotyping efforts, and on the development of tools to facilitate communication between bioinformatics and plant breeders.

The study of Spanish barley landraces though association mapping, linkage mapping and sequencing is a good example of the utilization of genetics and genomics to unravel the genetic control of adaptive traits, with the great help from draft reference genome of barley for the last two years. Specific adaptations to prevailing climate and diseases have been found and are already being exploited in breeding. Examples of progresses in the fields of genetic control of phenological responses and disease resistance will be discussed. The adaptation traits found in landraces are particularly useful now, because Mediterranean climates pre-figure climates expected in Europe and other regions and, therefore, can contribute to develop cultivars in a climate change scenario.

Session 1:
Genome structure, function and evolution

Tracking legume ancestral genome: insights from the comparative mapping of *L. angustifolius* gene-rich regions

Michał Książkiewicz[1], Sandra Rychel[1], Katarzyna Wyrwa[1], Anna Szczepaniak[1], Andrzej Zieleziński[2], Wojciech Karłowski[2], Bogdan Wolko[1], Barbara Naganowska[1]

[1]*Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 61-479 Poznań, Poland*
[2]*Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland*

Narrow-leafed lupin (*Lupinus angustifolius*) has been recently considered as a legume reference species. The progress in molecular biology methods considerably accelerated the studies on the *L. angustifolius* genomics. New genetic resources have been developed, including nuclear DNA libraries, a draft genome sequence, linkage maps with extensive gene-anchored markers, comprehensive transcriptome assembly and cytogenetic chromosome-specific landmarks. Here, we used the above-mentioned resources, together with those generated for other legume species, to identify and analyze *L. angustifolius* gene-rich regions (GRRs).
The *L. angustifolius* genomic BAC library was screened with several SSR- and gene-based probes, and clones carrying particular sequences were selected. To examine their hypothetical clustering in the genome, the clones were subjected to restriction enzyme DNA fingerprinting, followed by contig construction. Annotation of BAC-end sequences (BESs) allowed us to select clones for sequencing. BAC sequences and BESs were used to design PCR primers for the amplification of DNA isolated from the parental lines of the mapping population: 83A:476 and P27255. The new BES- and BAC-derived markers were localized on the *L. angustifolius* genetic map. When BESs and sequenced BACs were aligned to the narrow-leafed lupin genome draft assembly, they tagged ~ two hundred sequences in total; thus approximately for every second BES a corresponding scaffold was identified. Orientations of more than one hundred scaffolds were identified by paired BESs. The *in silico* detection of coding regions in scaffolds revealed that the average gene density was about 9-10 genes/100 kb of sequence. However, as many as 40 scaffolds were classified as GRRs, with an average gene density of ~ 20 genes/100 kb (and maximum up to 35 genes/100 kb). Such values are similar to those previously calculated for other two GRRs in narrow-leafed lupin. Using genetic linkage distances and consensus band size data, the ratios of physical to genetic distances were calculated for mapped scaffolds and BACs. Values obtained for gene-rich regions were about 100-170 kb/cM. The physical-to-genetic distance ratios supported the results of functional annotation, since the recombination frequency is positively correlated with gene density. Comparative analysis of *L. angustifolius* BAC and scaffold sequences revealed syntenic links to the GRR sequences of five sequenced legume species (*Medicago truncatula*, *Glycine max*, *Lotus japonicus*, *Phaseolus vulgaris* and *Cajanus cajan*).

Financial support: Polish Ministry of Science and Higher Education / National Science Centre project N N301 391939.

Development of the genetic diversity of triticale (× *Triticosecale* Wittm.) by the intergeneric crosses with goatgrasses (*Aegilops* spp.)

Michał Kwiatek, Maciej Majka, Jolanta Belter, Halina Wiśniewska

Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 61-479 Poznań, Poland

Fungal diseases of triticale (×*Triticosecale* Wittm.), such as leaf rust and powdery mildew have escalated recently. Introgression of effective resistance genes from wild species is a way to increase the genetic diversity of triticale. *Aegilops* spp. carry many resistance genes to biotic factors: rusts, powdery mildew, eyespot, and abiotic factors: drought and salinity. The goatgrass-rye amphiploids can be used as a "bridge" to transfer useful agronomic traits from *Aegilops* spp. to cultivated cereals, like triticale and rye. Chromatin transfer from *Aegilops* × *Secale cereale* to hexaploid triticale, determination of chromosomal structure in four subsequent generation of hybrids using molecular cytogenetics and identification of molecular markers linked to fungal disease resistance genes are main aims of our research. Four tetraploid species: *Aegilops biuncialis* Vis. (UUMM, 2n=4x=28), *Ae. ovata* Roth. (UUMM, 2n=4x=28), *Ae. kotschyi* Boiss. (UUSS, 2n=4x=28), *Ae. variabilis* Eig. (UUSS, 2n=4x=28), one diploid species – *Ae. tauschii* Coss. (DD, 2n=2x=14), five amphiploid forms of *Aegilops* spp. × *Secale cereale* and four subsequent generations obtained by reciprocal crosses between amphiploids and 5 triticale cultivars were used in this work. Genomic *in situ* hybridisation (GISH) was carried out to categorize the mitotic and meiotic (MI) chromosomes. Fluorescence *in situ* hybridisation (FISH) with pSc119.2, pAs1, 5S rDNA and 25S rDNA probes were used to differentiate particular chromosomes. FISH/GISH analyses allowed to identify chromosomal translocations between *Aegilops* genomes (intragenomic translocations) and chromosomal translocations between *Aegilops* and triticale genomes (intergenomic translocations). Differences in localization of the rDNA, pSc119.2 and pAs1 sequences between analogue subgenomes in diploid and tetraploid species (*Aegilops* spp.) and hybrids were detected. Molecular analysis were made in order to identify molecular markers linked to leaf rust and powdery mildew resistance genes. Hybrids with introgressed chromatin of *Ae. kotschyi*, *Ae. variabilis*, *Ae. ovata* and *Ae. biuncialis* carried molecular markers (*Lr37*+*Sr38*+*Yr17*) linked to leaf rust resistance genes. The marker for *Lr32* and *Lr39* was detected in hybrids with *Ae. tauschii* chromatin. Amplification products specific to *Pm13* marker (linked with powdery mildew resistance genes) were obtained in hybrids with *Ae. variabilis* chromatin.
Selected (*Aegilops* spp. × *S. cereale*) × triticale hybrids with translocations of *Aegilops* chromatin, that ensure an introgression of desirable agronomic traits, could be initial materials for breeding, aiming in triticale improvement.

Poster 1.1

Search of housekeeping genes in rhododendrons based on the analysis of orthologous genes in the model plant *Arabidopsis thaliana*

Jarosław Pławiak, **Małgorzata Czernicka**

Institute of Plant Biology and Biotechnology, Unit of Genetics, Plant Breeding and Seed Science, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Krakow, Poland

Housekeeping genes are known as widely used reference genes for quantitative real-time PCR (RT-qPCR). The transcription level of these genes should be constant in the cells of different tissues and under different conditions. So far, there has been no information on rhododendrons housekeeping genes useful for real-time PCR analysis. The aim of the study was to identify the housekeeping genes i.e. *act2*, *ef1, tua4, hsp70*, SAMDC, *tub9* which could be applied as reference genes for real-time PCR analysis to measure the relative expression of genes related to frost resistance. The multiple sequence alignment (MSA) was applied to compare DNA sequences from different plant organisms using as query *Arabidopsis thaliana* housekeeping gene data. As a result we compared from 80 to 130 genomic and mRNA sequences deposited in NCBI for each gene. Consensus sequences based on MSA allowed to localize at least two most conserved fragments within exons which were used for designing PCR primer pairs. The sequencing and bioinformatic analysis of five gene sequence fragments confirmed that they were really housekeeping genes i.e. *act2, ef1, hsp70, tua4, tub9*. The results pointed out that the conserved CDS sequence fragments of *Arabidopsis thaliana* allowed to identify earlier unknown *Rhododendron* housekeeping genes.

Poster 1.2

Prelude to 'Old World' lupin species evolution: comparative cytogenetic analysis of chromosome rearrangements

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During the course of plant evolution, multiple chromosomal rearrangements took place, in particular within legumes, which among many others are 'victims' of one or more rounds of polyploidisation. As a result, abundance of gene copies in genome allowed for increased mutation rate in their sequences, which led to the emergence of new genes or new gene variants, and in consequence to diversification within legumes. A good example are 'Old World' lupin species, comprising species with various basic and total chromosome numbers as well as species with different chromosome numbers but the same genome size and conversely, with the same genome size but different chromosome numbers. In the light of increasing role of lupin crops in agriculture, 'Old World' lupins are a valuable plant group for comprehensive analyses, aimed at developing more efficient methods (e.g. in plant breeding programmes) due to better understanding of plant genetics.
Cytogenetics is used to understand chromosome structure and genome evolution in many plants, including both monocots and dicots. Fluorescence *in situ* hybridisation (FISH) is a widely applied chromosome identification technique, useful for tracking chromosome changes and comparative mapping. Herein, to accurately identify chromosome rearrangements among lupins, the BAC-FISH method has been used. A set of BAC-derived chromosome markers of *Lupinus angustifolius*, describing representatives of 'Old World' lupins in the best way, has been used as probes for multicolour FISH. The analyses have been performed for four wild species: *L. cosentinii* (2n=32), *L. cryptanthus* (2n=40), *L. pilosus* (2n=42) and *L. micranthus* (2n=52). We have found that some clones used for chromosome identification in *L. angustifolius*, localized on the same chromosome, were also mapped on one chromosome in analyzed species. Moreover, the 'single-locus' *L. angustifolius* BACs have been dispersed along chromosomes of other species. This evidence of chromosome rearrangements has shed light on lupin complex evolution and/or speciation.
Our results will allow us to track chromosome rearrangements within the genus as well as learn mechanism behind those changes. Ideally, it would lead to design an evolutionary model of lupin species studied. Additionally, it would enable us to answer a question whether BAC-derived clone sequences of *L. angustifolius* can be reliable markers for chromosomes of different lupin species.

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Poster 1.3

Gene copy number variation in *Lupinus angustifolius* genome

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The era of whole-genome sequencing has revealed that gene copy number changes have important evolutionary, functional, and phenotypic consequences in plants. However, the methodology of determining copy number variants of genes (CNV) is not fully dependable. In the present study we compared three methods for the detection of CNV of eleven genes playing crucial roles in nitrogen fixation and fatty acid synthesis in *Lupinus angustifolius* (narrow-leafed lupin), a reference species for genomic studies within the genus *Lupinus*. First, we measured the copy number of analyzed genes using quantitative Real-Time PCR (qPCR). Obtained results were not consistent, probably due to variations in the amplification efficiency which affected the reliability of measurements. To overcome this drawback, we applied high-throughput Droplet Digital PCR (ddPCR) which provided precise and repeatable results. Simultaneously, to support/verify our molecular analyses, we used BAC clones from the *L. angustifolius* nuclear genome library, carrying sequences of analysed genes, as probes in fluorescent *in situ* hybridization (FISH). These BACs were localized in metaphase chromosomes and mapped in appropriate linkage groups by BAC-based genetic markers.
Gene CNV as a common form of genome natural diversity has enormous potential for model organism research, evolutionary biology, and crop science. Our results will contribute to further genomic studies in lupins, especially to get insight into genus evolution.

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Poster 1.4

Isoflavone synthase genes in *Lupinus angustifolius* L.

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Isoflavonoids - the important group of secondary metabolites are produced mainly by the legumes as the important products of their phenylpropanoid biosynthesis pathway. Isoflavone synthase (IFS) is a key enzyme in the branch of this pathway initiating the synthesis of these compounds. The enzyme is a polypeptide chain of about 520 amino-acids.
Six BAC clones containing the isoflavone synthase encoding fragments were selected by screening the BAC library of *Lupinus angustifolius* cv.Sonet. Three of these clones have been sequenced. The nucleotide sequences of IFS genes and its surroundings were determined. Each of three BAC clones sequenced revealed the presence of distinct IFS sequence. These sequences are not identical, however they reveal high degree of homology on both nucleotide and amino-acid level. The comparison of *Lupinus angustifolius* IFS sequences with the respective sequences from other plant species shows the general high diversity (the identity within the range 36 – 92% was found). The analysis of expression of IFS encoding genes in various organs of *Lupinus angustifolius* was performed using RT-PCR technique. The levels of transcription appeared to be relatively high particularly in roots and root nodules. Our results strongly suggest that isoflavone synthase is encoded in *Lupinus angustifolius* by small multigene family consisting of at least three members. All of the analyzed BAC clones were localized by FISH-BAC within the chromosomes of *Lupinus angustifolius*. In order to prove that analyzed sequences are IFS encoding genes, the theoretical models of enzymes were constructed using Swiss PDB Viewer. The models reveal all features characteristic for functional enzyme.

Poster 1.5

Molecular control of flowering time and vernalization in lupins

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Narrow-leafed lupin (*Lupinus angustifolius* L.) is a grain legume crop valuable for animal feed which is gaining recognition as a potential human healthy food. Due to global climate warming, the optimal time frame for sowing of vernalization-dependent lupins is being reduced, therefore thermoneutrality and short vegetative phase should be the most important characteristics in selection of new cultivars.
It has been shown that some varieties of *L. angustifolius* are early flowering and do not require vernalization treatment, that results in considerable reduction of time from sowing to the start of flowering. A *Ku* gene, which removes the requirement of vernalization and advances flowering by about 2-5 weeks, has been already described. Lupin lines having this gene are thermoneutral and less responsive to photoperiod, i.e. do not need long day length to initiate the flowering phase. Recent studies on model species have shown that, in the induction of flowering, FT gene homologues play key roles. Our analyses have demonstrated that very specific alteration in the FT gene sequence is responsible for an early flowering phenotype in *L. angustifolius*. We have obtained sequences of both alleles from a thermoneutral variety (Sonet) and a vernalization-dependent wild line. Based on this difference, genetic markers have been developed and localized on the linkage map.
The studies on flowering time in white lupin (*L. albus* L.) revealed polygenic control of this trait, in contrasts to *L. angustifolius,* where, as shown above, early flowering is conferred by a major gene, *Ku*. Sequences of the genes known to be associated with flowering regulation in model species were subjected to comparative analysis with *L. angustifolius* genome scaffolds, *L. albus* transcriptome and other legume EST and Uni-Gene reads. To survey nucleotide polymorphism between early and late white lupin lines, PCR primer pairs anchored in several homologues were designed and amplified products were sequenced. Polymorphic loci were identified in every studied gene. Segregation of these markers will be tested using the population derived from a cross between cv. Kiev Mutant and an Ethiopian landrace P27174, containing one hundred and ninety-five F_8 recombinant inbred lines. Localization on the genetic map of *L. albus*, carrying 220 AFLP and 105 gene based loci, will allow us to estimate the linkage between new markers and flowering time.

The research was funded by the Ministry of Agriculture and Rural Development, project no. 39.

Poster 1.6

Genetic mapping of genes underlying quinolizidine alkaloid biosynthesis in the narrow-leafed lupin (*Lupinus angustifolius* L.) genome

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Quinolizidine alkaloids (QA) are secondary metabolites characteristic to the species of the genus *Lupinus*, including narrow-leafed lupin (*Lupinus angustifolius* L.). Due to their toxicity and bitter taste, low alkaloid content in seeds is especially important for propagation of lupins in animal feeding and human consumption as a valuable protein source. The biochemical synthesis pathway of lupin alkaloids is partially known, however genes underlying QA biosynthesis still remain poorly investigated. Up until now, only one recessive gene *iucundus* controlling total alkaloid content has been used in the narrow-leafed lupin breeding programs. It has also been confirmed as the main gene affecting the alkaloid content in our previous QTL analyses. Unfortunately its molecular function has not been yet determined.
On the basis of the transcriptome sequencing experiment (RNA-seq) results several genes assumed to be involved in the QA biosynthesis pathway have been identified. The main aim of the presented study was a genetic mapping of selected genes underlying QA biosynthesis in the narrow-leafed lupin genome, using 83A:476 x P27255 mapping population. Knowlege of these genes location in relation to the position of the low alkaloid content gene *iucundus* may result in unraveling *iucundus* gene function. In a long term it may also have more practical application allowing the design of markers linked to the low alkaloid content useful in the narrow-leafed lupin marker assisted selection.

Poster 1.7

Genetic mapping and QTL analysis of stem parameters and lodging in Carneval × MP1401 pea (*Pisum sativum* L.) mapping population.

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Legumes are important plants for agriculture. They have a symbiotic relationship with bacteria that contain nitrogenaze- enzyme responsible for biological nitrogen fixation. Legumes are used in crop rotations to replenish the nitrogen in the soil. They are also a good source of protein for food and feed. Among grain legumes the most widely grown in Europe is dry pea (*Pisum sativum* L.) One of the main constraints in pea cultivation is it's susceptibility to lodging. Lodging may cause severe yield loss and contributes to the yielding instability of the pea.
Our previous research suggests that stem parameters (stiffness, endurance, and diameter and stem wall thickness) are correlated with lodging susceptibility. Stem parameters, lodging susceptibility and plant height were tested in a Carneval x MP1401 RIL population. High-density genetic maps are necessary for comparative genomics and QTL mapping. Genetic map for Carneval × MP1401 RIL (recombinant inbred lines) population was published (Taran et al. 2003). It consisted mainly of AFLP markers. New polymorphic markers (STS, SSR, isozymic) were found and mapped in order to obtain a denser genetic map. New map was constructed using JoinMap 2.0 software. It consists of 226 markers (203 AFLP, 9 STS, 12 SSR, 1 isozymic, 1 SCAR) separated into 15 linkage groups and spans approximately 596 cM.
Both parents of Carneval × MP1401 mapping population are semi-leafless and have short internodes. Carneval is lodging resistant, MP1401 is lodging susceptible. Three stem heights were analyzed: lower (2nd-4th internode), middle (9th-10th internode) and upper (below the first generative node). Lodging was estimated in 3 periods (before flowering, after flowering and before harvesting) and on a 9-point scale (1- susceptible, 9- not susceptible). QTL analysis was performed with QTL Cartographer 2.5 software. Data from four years were analyzed. Statistical analysis revealed that lodging is negatively correlated with stem diameter and endurance in the middle of the stem height. 46 loci were found. Results were no consequent across the years. Measured traits are strongly affected by the environmental conditions. Markers for selection within marker-assisted breeding were identified to raise the efficiency of germplasm selection.

The study is supported by National Multi-Year Program "Improvement of domestic sources of plant protein, their production, economy and feeding technologies".

Poster 1.8

Photosynthetic capacity and components of water and nitrogen efficiency in selected cultivars of Polish and German collections in field pea (*Pisum sativum* L.)

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The worldwide competition for protein resources as well as dependence of European countries on imports of soybean meal and global ecological aspects have led to increasing demands for home-grown and sustainable protein sources such as grain legumes used in EU mostly for livestock feeding. The crude protein content varies considerably between legume genotypes and species, especially when those are, in practice, grown in diverse environmental habitats. This variation seems to demonstrate a potential for further pea improvements using more efficient breeding and selection strategies.
The present research project was aimed to search for potentially innovative, modern selection techniques that could be utilized in the breeding strategy. We assume, the molecular tools could especially be used either to evaluate or select breeding materials and cultivars for some complex physiological traits (e.g. photosynthetic capacity and water and nitrogen use efficiency). In the study done under controlled conditions in vegetative phase, variance and co-variance of the traits was examined among cultivars of Polish and German collections grown under optimal water and nutrient supply. Chosen molecular markers, linked with selected traits, were tested in the materials. Genotypic differences were significant for the photosynthesis rate (P_n), transpiration rate (Tr); stomatal conductance(g_S), carboxylation efficiency (P_n/C_i), amount of transpired water, nitrogen content in plant biomass (%N) and nitrogen utilization efficiency (NUtE). Close positive correlations between P_n, g_S and P_n/C_i were found at the growth stage. Positive correlation exits between water use efficiency (WUE) and plant biomass. The most N-effective cultivars were the cvs. Santana, Madonna and Lasso. Principle Component Analysis distinguished the cvs. Alvesta, Lasso, Madonna, Navarro and Salamanca as the most efficient in WUE and NUtE.
The data suggest that the examined physiological components might be crucial for pea yielding. As a result of this study, the proposed set of molecular markers is expected to be utilizable in pea selection that deals with increased protein content and improved pea adaptation to sustainable agriculture.
The study is supported by the Opening National and Regional Programmes for Transnational Collective Research between SME Associations and Research Organisations (CORNET 15th) "Innovative Protein Products from Sustainably Grown Legumes for Poultry Nutrition (ProLegu)".

Poster 1.9

Variability of European grass pea accessions (*Lathyrus sativus* L.) for morphological traits and chemical composition of seeds.

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Investigations have usually been focused on the few staple crops, while relatively little attention has been given to underutilized or neglected species. To such crops belong some species of the genus *Lathyrus*. It covers 187 species and subspecies that are found in both the Old World and the New World. However, only one species – grass pea (*Lathyrus sativus* L.) is widely cultivated as food crop, while other are cultivated to a lesser extent for booth food and forage. Grass pea, like other grain legumes, is cultivated mainly in consideration of high protein content in the seeds. In comparison to another legumes has a high yielding potential on low fertilization level and is also very tolerant to drought conditions. Grass pea has been identified as an important source of novel genes for use in grain legume breeding programs, not only for abiotic but also for biotic stresses resistance. Unfortunately wider utilization of grass pea is limited by some antinutritional factors, especially neurotoxins, causing lathyrism both in animals and in humans.
The aim of investigations was the determination of morphological traits in the field trials in connection with a determination of the content of nutrients and some antinutritional factors in *Lathyrus sativus* seeds. The initial material for the study covered accessions of grass pea originated from different parts of Europe. Apart from observed differentiation in terms of morphological traits (branch and leaf characters, plant growth habit, flower colour, pod shape, seed shape, seed coat colour, seed size and 100-seed weight), particularly important was broad variability of chemical composition of seeds. Accessions with high protein and fat content, characterized by valuable fatty acid profile were indentified. A great differentiation was also observed for fiber content and minerals as: potassium, copper, zinc, iron and manganese. The most important factor limited use of grass pea for food production and feeding purposes is a content of anti-nutritional compounds in seeds. Most of the reported works on grass pea improvement has been done on the reduction of β-ODAP content in the seeds. This anti-nutritional compound in prolonged or excessive consumption of grass pea seeds lead to drastic paralytic disease known as "lathyrism", manifesting for example as paralysis of the leg muscles. The evaluated accessions of *Lathyrus sativus* contained fairly low level of β-ODAP. Average content of this toxin was amounted to 847 $mg \cdot kg^{-1}$DM. Large differentiation, however, was noted between accessions and lines with decreased β-ODAP content were indentified. Average content of tannins was near 3,8 $mg \cdot kg^{-1}$DM. The lowest level of this antinutritional factor was noted in accessions of Polish origin.

Poster 1.10

Development of molecular probes enriched for sequence repeats as a tool for *Festuca pratensis* chromosome mapping

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Species belonging to the *Festuca-Lolium* complex are important forage and turf species and as such, have been studied intensively. However, their out-crossing nature and limited availability of molecular and physical markers make genetic studies difficult. Robust molecular techniques such as molecular cloning of DNA sequences, next-generation sequencing, *in situ* hybridization and bioinformatic analyses are important tools to be applied in understanding the dynamic changes in the nuclear genome architecture. To study *F. pratensis* genome organization (architecture) we have prepared a genomic library from the *F. pratensis* nuclear DNA in a cloning vector. The nuclear DNA was digested completely with *Hind*III restrictase and the fragments were ligated with pBluescript KS(+) plasmid and cloned in XL1Blue MRF' *E. coli* strain. The 654 clones have been isolated. They represent the most abundance sequences in the genome and their DNA complexity was analyzed by restriction polymorphism and molecular size. The DNA library representing sequences the most frequently present in the *F. pratensis* genome will be used for the genome characterization and chromosome mapping. The library provides wide accessible molecular probes for *in situ* hybridization to identify the *F. pratensis* chromosomes and their arms and is expected to have a major impact on the *Festuca* and *Lolium* genomics.

Poster 1.11

Zooming in on the *Brachypodium – Festuca*/*Lolium* complex chromosomal mapping

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Over the past decade, expressed sequence tags (ESTs) combined with high resolution genetic mapping, BAC clones libraries, *in situ* hybridization and subsequently bioinformatic analyses have been shown to be advantageous in increasing our knowledge about grass genome evolution and updating the original 'crop circle'. This circle, originally proposed by Graham Moore, concerns mainly the key importance cereals, such as rice, maize and sorgo but also *Brachypodium distachyon* (Brachypodium; $2n=2x=10$), which is a model organism to study the nuclear genome organization in grasses.

In this work we physically mapped by FISH Brachypodium-originated BAC-based probes to chromosomes of two forage grass species, *Festuca pratensis* ($2n=2x=14$) and *Lolium perenne* ($2n=2x=14$). Since physical maps of Brachypodium are well developed, preliminary comparative chromosome alignments of Brachypodium with *F. pratensis* and *L. perenne* regions have provided information about number and distribution of syntenic segments in regard to relevant Brachypodium chromosome arms. These advances mark the first step towards using the Brachypodium genome as a reference physical map to provide evolutionary annotation to *Festuca* and *Lolium* chromosomes.

Poster 1.12

Analysis of *Ppd* alleles structure in triticale hybrid plants with different flowering time

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Triticale (X *Triticosecale* Wittmack) is an intergeneric hybrids combining in one organism quality and yield of wheat and resistance to biotic and abiotic stresses of rye. Triticale is relatively novel cereal species and because of that fact the physiological and molecular mechanism of certain traits has not been accurately described so far.

One of the most important trait in cereals is regulation of flowering time as well as insensitivity on length of the day. One of the most important factors determining this trait on molecular level is composition of *Ppd* genotype in plant genome.

In present research we analyzed triticale hybrids derived from [Clever × (Amilo × *Dasypyrum villosum*)] × Nadobna cross combination. Plants originated from this combination have divided into two groups presenting different flowering time. The difference in flowering time between extreme objects from these two groups noticed in field experiment was 10 days.

Early and late genotypes have been crossed and flowering time of each plant of F_2 segregating population was recorded. The DNA from parental forms has been isolated and *Ppd* alleles (*Ppd-A1*, *Ppd-B1, Ppd-D1*) presence have been determined by means of sequence specific molecular markers. Obtained PCR products have been sequenced and comparative analysis with products specific for wheat was performed. In presented research high level of similarity between *Ppd* alleles from wheat and triticale genomes has been shown, however some alteration have been identified and described. Presented results can increase our knowledge about determination of flowering time in triticale.

Presented research are carried out within the framework of project "Development of novel triticale genetic sources on the basis of wide crosses" funded by Polish Ministry of Agriculture and Rural Development.

Poster 1.13

Genetic improvement of triticale by distant crosses with *Aegilops tauschii* × *Secale cereale* amphiploid forms in order to transfer the leaf rust resistance genes

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Widening the genetic variability is important in the case of triticale (× *Triticosecale* Wittm.), as it is a fully synthetic species with a narrow range of genetic variation. Therefore, the distant crossing is an excellent method which enable to generate new forms of triticale with chromatin introgression i.e. from wild species of the *Aegilops* sp. genus. The main aim of this research is effective usage of *Aegilops tauschii* × *Secale cereale* (DDRR, 2*n*=4*x*=28) amphiploid forms carrying,*Lr22a*, *Lr32* and *Lr39* leaf rust resistance genes used to transfer them into triticale cultivar "Bogo" (AABBRR, 2n=6x=42) to widen the genetic diversity. There was performed identification of molecular markers specific to chromosomes bearing genes determining resistance to leaf rust and markers combined with these genes as well as *in situ* hybridization analysis of metaphase of mitosis. In the experiments four highly repeated sequences were used as probes: pTa 794 (5S rDNA), pTa71 (35S rDNA), pSc119.2 and pAs1 to distinguish chromosomes. First FISH experiments were performed for the ancestral species of the hybrids: *Aegilops tauschii* Coss. (2*n*=2*x*=14, DD) and triticale cv. "Bogo" (2*n*=6*x*=42, AABBRR) to facilitate the exact identification of D- genome chromatin introgression. GISH was used to differentiate D- genome chromatin from triticale. The next step concerned analyses of given *Aegilops*/triticale hybrids and select ones with D- chromatin introgression, that could enrich the triticale in resistance to leaf rust. This study confirmed the stable heritage of 3D chromosome pair from F_1 to BC_3F_1 generation of (*Ae. tauschii* × *S. cereale*) × triticale hybrids, presence of additional chromosomes 2D or 3D in hybrid forms from BC_1F_1 to BC_4F_1 generation and also 3DL_2DS.2DL in BC_4F_1. The molecular marker analysis showed that occurrence of alien chromatin from *Ae. tauschii* is combined with transferring genes determining resistance to leaf rust. The results indicate that using amphiploid forms as a bridge between wild and cultivated forms can be considered as a successful way of D-genome introgression carrying leaf rust resistance genes into triticale. Selected (*Ae tauschii*. × *S. cereale*) × triticale hybrids with translocations of *Aegilops* chromatin, that ensure an introgression of desirable agronomic traits, could be initial materials for breeding, aiming in triticale improvement. This approach will increase resistance to fungal infection and improve agronomic value of triticale.

Poster 1.14

Analysis of influence of novel dwarfing gene presence on GA 3-oxidase gene expression in triticale (X *Triticosecale* Wittmack)

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Gibberellins (GAs) are one of the major phytohormones that regulate various aspects of plant developmental processes. One of the most important function of gibberellins is stimulation of stem elongation. The gibberellins class comprise many different compounds. However, only few of them show biological activity in plant tissues. The process of bioactive gibberellins biosynthesis is a complex pathway including seven different enzymes. The major role in this process is played by oxidases. The GA 3-oxidase (GA3ox) is the enzyme responsible for direct transformation of gibberellins GA_9 and GA_{20} (non-bioactive) into GA_4 and GA_1 (bioactive). The majority of genes encoding enzymes involved in GAs biosynthesis have been described so far. The novel research revealed that some of dwarfing genes known for many years in cereals are the genes encoding altered forms of enzymes involved in bioactive GAs biosynthesis.
The objective of presented research was comparative analysis of expression of the GA3ox encoding gene between triticale plants containing novel dwarfing gene and plants of tall control line. The influence of exogenous application of gibberellic acid (GA_3) on analyzed gene expression in both lines have been estimated as well.
Obtained results did not revealed meaningful differences between dwarf and tall analyzed line in GA3ox gene expression. Moreover, we shown that exogenous application of GA_3 caused did not cause alteration of analyzed gene expression as well. These results suggest that in analyzed triticale genotypes molecular activity of dwarfing gene is linked to another step of GAs biosynthesis pathway or to degradation of their bioactive forms. Because of that fact subsequent analysis of expression for genes encoding other enzymes (especially GA20ox and GA2ox) are necessary.

Poster 1.15

Assessment of morphological and molecular diversity between rye NILs differing in dwarf gene.

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Plant resistance to lodging is primarily connected with the morphological characteristics of plants, stalk anatomy and structure of the root system. Plant breeding for resistance to lodging is difficult because it is a quantitative trait controlled by multiple genes with significant impact on the expression characteristics of the environment. A number of researchers look for morphological traits correlated with lodging, which could be used in the selection of resistant genotypes. It is considered that the most associated with resistance to lodging is the height of the plants. Significant height reduction in cereals is caused by dwarfing genes, which are divided into two groups: gibberellin (GA) insensitive (unresponsive to exogenously applied GA) and sensitive. In main cereals, many dwarfing genes have been incorporated into European or American germplasm and used in cultivar development.
The aim of this study was to detect a morphological and molecular polymorphism between near-isogenic lines (NILs) of rye varied in terms of height. Experimental material was developed from the RIL population S120×S76. The dwarf mutant-plants appeared in the S_4 generation which was due to the presence of the recessive allele. Three pairs of sublines (tall and dwarf variant in each pair) were selected from the segregating heterozygous high plants of S_5 generation. NILs were characterized in terms of: plant height, number of internodes, length of the second internode and length of the peduncle, number of spikes per plant, main spike length, number of spikelets per spike, number of grains per spike and kernel weight. The gibberellic test executed in hydroponic experiment showed sensitivity to GA of both dwarf and tall forms of NILs. Two molecular techniques were used to assess genetic diversity of each pair of NILs and to find markers for dwarf gene. There were 39 908 DarT-seq-*in silico* markers obtained and 92-331 of them were polymorphic, depending on the NIL pair. Only one polymorphism was common for all 3 pairs of NILs. Among 14 888 DarT-seq-SNP markers 15 were joined by 3 pairs of sublines. Out of 1 242 RAPDs tested, 5 were polymorphic for 3 NIL pairs.

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Poster 1.16

Comparative mapping of major restorer genes in two sterility-inducing cytoplasms of rye.

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Breeding of hybrid cultivars of rye is becoming year to year more popular. Generally, the breeding of rye hybrids is based on the Pampa sterility-inducing cytoplasm (CMS-P), but the use of an alternative type of CMS in practice is strongly recommended. One of potentially applicable sources of CMS in rye is the C cytoplasm (CMS-C). The genetic background of restoration male fertility in both mentioned sterility-inducing cytoplasms is still unclear. During the last decade effective restorer genes for CMS-Pampa and CMS-C systems were localized on the long arm of the chromosome 4R. It is still unclear, if these restorers are allelic or only linked genes. This work was aimed at the application of different molecular markers for comparative mapping of both loci of interest.
Two mapping populations were developed. The population with the Pampa cytoplasm was obtained by pollination of male sterile inbred line 541P with pollen of one plant randomly chosen from IRAN IX population. The second population was derived from the interline cross between male sterile 544C and restorer Ot0-20. Both populations were phenotyped and genotyped in advanced generations (BC5F2). Comparative mapping with the use of PCR-based markers as well as Diversity Arrays Technology (DArT) revealed localization of major restorer genes for both CMS-systems in close vicinity on the 4RL chromosome.

This work was financially supported by Polish Ministry of Agriculture and Rural Development (dec. HOR hn - 801-5/14)

Poster 1.17

Development of a carrot genotyping panel based on *DcSto* insertion polymorphisms

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Transposable elements (TEs) are one of the important constituents of the genome of all living organisms. Their mobilization leads to genetic variability. Being a significant factor determining genetic variation, they are potentially a good source of molecular markers. Recently, the present authors have characterised 12 families of *Stowaway* MITEs (*DcSto*) – short non-autonomous DNA transposons found in the carrot genome. These elements are preferentially associated with genic regions. There are more than 4000 copies of *DcSto* elements in the carrot genome. The number of copies ranges from 55 for a low-copy *DcSto*6 family to approximately 1000 for the most prolific *DcSto*10 family and varies amongst individual chromosomes (from 319 on chromosome 9 to 581 on chromosome 1). At least 15% of characterised elements are inserted into the transcribed regions. An analysis of the exact localization of insertion in a reference genome allowed identification of *DcSto* copies inserted into short introns (up to 3 kbp), free from any other repetitive sequences, and designing 209 site-specific primers anchored in adjoining exons. The development of a panel for genotyping carrot was preceded by the selection of 10 to 20 site-specific primers allowing amplification of insertion sites evenly distributed over each chromosome. Cultivated and wild carrot accessions were tested for the occurrence of different allelic variants. The outcome of the initial study shows a considerable *DcSto* insertion polymorphism between and within the studied populations. Wild accessions, compared to the cultivated ones, are characterised by infrequent presence of insertions within the amplified introns. The panel under development may contribute to lessening of the cost- and time-consumption of genotyping the carrot by means of applying a simple PCR technique allowing detection of polymorphism of the *DcSto* insertions.

The research was funded by Ministry of Agriculture and Rural Development, project HORhn078/PB/34/14, awarded to Dariusz Grzebelus.

Poster 1.18

Distribution of low copy LTR retrotransposons in the genome of carrot (*Daucus carota* L.)

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Long Terminal Repeat (LTR) retrotransposons are Class I transposable elements mobilized via a 'copy and paste' mechanism utilizing an RNA intermediate. *gypsy* and *copia* superfamilies of LTR elements are particularly widespread in the plant kingdom and frequently constitute a major fraction of repetitive DNA in plant genomes. Families of LTR retrotransposons can reach very high copy numbers, usually occupying pericentromeric heterochromatin regions. However, low copy number families of LTR retrotransposons are more frequently present in gene-rich regions. Insertions of LTR elements were reported as responsible for rearrangements resulting in phenotypic variants of the host species, some of them being important for agricultural production. Here, we present preliminary results on the host intra-specific diversity with respect to two *gypsy* and two *copia* LTR families. Three of the reported retrotransposons, namely *DcG01*, *DcG02* and *DcC02*, were identified as single copy insertions in the carrot reference genome. *DcC01* was not present in the reference genome; it was found in other carrot accessions following PCR amplification of the region where *DcG01* was located. Insertions of *DcG01*, *DcC01* and *DcC02* were in genic regions, less than 2 kb from adjacent genes, while *DcG02* was inserted in the third intron of a gene coding for a putative calcyclin binding protein. Resequencing data from 27 accessions of wild and cultivated carrots of different origin revealed that all four families showed patchy distribution. Their presence or absence in individual genomes could not be attributed to their geographic origin and/or breeding history. Distribution of *DcG01* was further analyzed in a collection of 88 accessions, representing genetic diversity in cultivated and wild *D. carota*, by PCR amplification of regions encoding *RT* and *gag* domains. Products confirming presence of *DcG01* copies were detected only in 21 (24%) of accessions. PCR amplification of the original *DcG01* insertion site showed presence of the element in that site in nine accessions of cultivated carrot. In the collection of carrot accessions we found four other insertion sites of which three were in close proximity to genes and in each case the *DcG01* insertion site was unique to the accession in which it was originally identified.

The research was supported by the Polish Ministry of Science and Higher Education fund for statutory activity of the University of Agriculture in Krakow.

Poster 1.19

Genome-wide analysis of carrot miniature inverted repeat transposable elements (MITEs)

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Transposable elements constitute a large fraction of plant genomes and strongly affect evolution of genes and genomes. MITEs are characterized by small size (>700), presence of terminal inverted repeats (TIRs), and high copy number. They preferentially insert within genic regions, which may influence structure and/or expression of adjacent genes. Previously, two groups of carrot MITEs, *Tourist*-like *Krak* and *Stowaway*-like *DcSto* elements were described. They were related to *PIF/Harbinger* and *Tc1/mariner* superfamilies of DNA transposons, respectively. Elements of both groups showed chromosomal distribution within euchromatic regions and high level of insertion polymorphism, possibly indicating their recent mobilization. Here, sequences of all members of *Krak* and *DcSto* groups were retrieved from a reference carrot genome with TIRfinder, a bioinformatic tool for MITE mining. We used TIR sequences as a mask for elements search, to identify only copies carrying complete TIRs. From a total of 498 *Krak*-like elements, 252 copies grouped within nine separate clusters (named *DcK1* to *DcK9*). The most numerous group, *DcK1*, comprised 152 copies belonging to the previously described *Krak* family. Elements of other groups represented eight new families of carrot *Tourist*-like MITEs, with sequence similarity to members of *DcK1* family and their putative autonomous counterparts, *DcMaster*s, limited to TIR regions. 4112 out of 4208 copies of *DcSto*s were classified into 12 families (*DcSto1* to *DcSto12*), including nine families described previously. Unclassified elements were most likely ancient copies of MITEs, too divergent to be classified based on a commonly accepted 80-80-80 criterion. Analysis of abundance and distribution of 21 MITE families at the chromosome level showed similar pattern of genes and MITEs density, with no MITEs within and near centromeres, confirming their preferential insertion into genic regions. 49 to 67% copies of all but one MITE families were located within 2kb to a nearby gene and 7 to 31% of them, depending on the family, were located within transcribed regions.

The research was supported by the Polish Ministry of Science and Higher Education fund for statutory activity of the University of Agriculture in Krakow.

Poster 1.20

4C-seq data processing, normalization and differential analysis of chromosomal contact profiles in *Arabidopsis thaliana*

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Numerous studies indicate that long-range chromosomal interactions contribute to gene and genome regulation. It was however not until the development of the chromosome conformation capture (3C) technology that the widespread role of chromosomal interactions became clear. One of the 3C-based methods is 4C-seq. This method provides information on physical interactions between a known fragment of interest and the rest of the genome. 4Cseqpipe is a computational analysis pipeline providing support for the analysis of 4C-seq experiments (van de Werken et al., 2012). It includes sequence extraction, mapping, normalization and the generation of high resolution contact profiles around the "viewpoint". To our knowledge, it has been used only for mammalian genomes, which happen to have different characteristics than a plant like A. thaliana. For this reason we propose a 4C-seq data processing schema partially based on tools commonly used for NGS data analysis and we show its application for four data sets obtained using the Arabidopsis FLC locus as a viewpoint. FLC serves as a very useful model system for epigenetic studies, because its expression can be modulated easily by changing growing conditions or by using mutants in various pathways. Concentrating on inter-chromosomal contacts, we present considerations concerning sources of data bias resulting from the distribution of A. thaliana genomic restriction sites and propose a data normalization method. Finally, we use statistical functional data analysis (Ramsay and Silverman, 2012) to find differences between contact profiles obtained for different samples.

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Session 2:
Stress response mechanisms

Oral presentation

Unknown mechanism of plant reaction to drought: changes in surface charge and acidity of roots.

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Huge amount of papers describe plant response to drought, however information on reaction of plant roots surface charge properties to drought conditions is very rare despite they are important for amount and ratio of cations uptake by plants. Since other stresses induce changes in roots surface charge properties, we hypothesized that drought does this also. Indeed, changes in CEC and acidity of roots of some cereal plants taken from pot (soil drought at water potential pF=3.5) and hydroponic (osmotic stress induced by mannitol) experiments determined using back-titration method certified this hypothesis. Both stresses applied at tillering stage caused down to fivefold decrease in CEC and up to tenfold increase in acidity of plant roots in various barley varieties.
Surface charge properties of nonstressed roots did not differentiate drought resistant and drought tolerant plants and varieties, however the intensity of roots reaction to the stresses seemed to be higher for drought sensitive plants. This newly presented mechanism of plant reaction to drought indicates that nutrients uptake by plants can be severely limited and relative uptake of polyvalent cations (aluminium or heavy metals) may increase in some cases causing additional toxicity. This may serve as additional explanation of plant growth and yield limitation in dry environments.

Oral presentation

Platform for analysis of barley pri-miRNA expression reveals a new mechanism of drought induced regulation of miRNA 444.3a level

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MicroRNAs (miRNAs) are small RNA molecules, usually of 21 nucleotides in length, which regulate gene expression through driving the target mRNA cleavage or repressing its translation. In this study, we have prepared RT-qPCR based platform for analysis of 140 primary micro RNAs (pri-miRNAs) expression level in barley. The platform was used for describing the relative expression level of the 140 pri-miRNAs in minor (30% Soil Water Content) and severe (20% SWC) drought treated plants, as well as after rehydration and in optimally watered plants. The drought stress modificated expression levels of the 140 pri-miRNAs were grouped into three categories: i) unchanged, ii) upregulated, iii) downregulated. We have compared the pri-miRNA expression profiles to mature miRNA levels obtained with Illumina deep sequencing of small RNAs. Northern hybridization was used to confirm the deep sequencing reads for chosen miRNAs. This method was also used to determine pre-miRNA level. An example of drought regulated molecule is miRNA444.3a. Its expression is downregulated at the level of miRNA, however the pri-miRNA444.3 level increases. This suggests a drought-induced pri-miRNA444.3 processing inhibition. A unique for miRNA444 family is the gene structure, where mature miRNA is separated from its miRNA* by an intron. The intron is obligatory to be removed to continue miRNA maturation. This allows for drought induced regulation of miRNA444.3 at the transcriptional level. The intron contains transcription start site proceeded by promoter where we discovered drought induced responsive elements. Drought makes the expression of transcript starting in the intron which is not able to give mature miRNA. The PARE (Parallel Analysis of RNA Ends) libraries screening allowed us to identify a target mRNA for miRNA444.3a which is MADS-box class II transcription factor. To sum up, we provide data describing changes in the expression profile of miRNAs, pre-miRNAs and pri-miRNAs in control and minor to severe drought conditions, as well as after rehydration. We conclude, that during drought stress the levels of miRNAs are regulated transcriptionally and also at the step of pri-miRNA processing (posttranscriptional regulation). Work sponsored by POLAPGEN-BD UDA.POIG.01.03.01-00-101/08 „Biotechnological tools for breeding cereals with increased resistance to drought”, subject 20: "The role of micro RNA in regulation of mechanisms leading to drought adaptation in plants"; Innovative Economy Programme 2007-2013, subject „Biological progress in agriculture and environment protection”.

Oral presentation

Heat stress responsive microRNA inhibit tillering in barley

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MicroRNAs are small RNA molecules, usually 21 nt long, that regulate gene expression through target mRNA cleavage or repressing its translation. MicroRNAs function as regulators of plant development and response to environmental stresses. Heat stress is one of the major abiotic factors that can induce severe plant damages leading to a decrease in crop plant productivity.
We have analyzed barley microRNAs expression profile under heat stress (2-weeks old barley plants exposed 24 h to high temperature) and compared them to microRNAs form barley plants grown under control conditions. We found 15 and 7 microRNAs which are up- and down-regulated, respectively. The most profound up-regulation was found for miRNA5048a, miR165a, and microRNA444.1 a2. The last one targets transcription factor (TF) that belongs to the MADS-box TF class II. Its rice ortholog OsMADS57 is a positive regulator controlling tiller outgrowth. MADS57 binds to CArG motif present in rice *Dwarf14* gene promoter and represses the expression of *D14* negative regulator of the tillering.
Alongside the elevated level of the barley mature miR444.1a2 during heat stress, we observed dramatic decrease of its target mRNA *HvMADS57* and inhibition of tillering in heat stressed plants suggesting the existence of a similar mechanism controlling tillering in barley as in rice plants.
To elucidate interplay between heat stress regulated microRNAs and their targets we carried out barley transcriptome sequencing (RNA-seq). A miRNAs/target genes network in response to heat stress in barley will be discussed in context of barley development.

The work was supported by the European Regional Development Fund through the Innovative Economy for Poland 2007–2013, project WND-POIG.01.03.01-00-101/08 POLAPGEN-BD "Biotechnological tools for breeding cereals with increased resistance to drought".

Oral presentation

Arabidopsis protein phosphatase 2C ABI1 regulates type III ACS protein turnover

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Arabidopsis thaliana type 2C protein phosphatase ABI1 (ABA-Insensitive 1) is a key protein and a negative regulator of abscisic acid (ABA) signaling. ABA plays significant role in plant growth, development and stress response. Numerous data indicate that ABA signaling is strictly connected with other phytohormone pathways e.g. ethylene (ET) pathway. Our previous studies showed that ABI1 is involved in ethylene biosynthesis by regulation of type I 1-aminocyclopropane 1-carboxylate synthase (ACS) protein stability. ACSs are pivotal enzymes during ethylene biosynthesis and are strictly regulated at both transcriptional and posttranscriptional levels. The stability of ACS isoforms is controlled by reversible phosphorylation/dephosphorylation events. ABI1-mediated dephosphorylation of type I ACS results in their rapid degradation by 26S proteasome. The analysis of ABI1 protein complexes using mass spectrometry revealed that a member of type III ACS - ACS7 is the ABI1 interacting partner. The *in vitro* pull-down assay confirmed the interaction between ABI1 and ACS7. To investigate whether ABI1 is involved in a control of ACS7 stability we performed *in vivo* degradation assay with and without proteasome 26S inhibitor – MG132. In extracts isolated from light-treated *ABI1* knockout plants, ACS7 remains stable, but in wild type extracts we observed significant reduction of the ACS7 protein level at 6 hour time point. These observations suggest that ABI1 affects ACS7 turnover only in light condition. In darkness, the level of ACS7 protein remains stable in WT and *abi1td* extracts. These results are consistent with the reduction of ethylene biosynthesis in light conditions. Our results indicate a new role of ABI1 protein phosphatase - targeting for degradation via ubiquitin-dependent proteasome 26S pathway.

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Poster 2.1

Involvement of genes encoding ABI1 protein phosphatases in the response of *Brassica napus* L. to drought stress

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Understanding the biological role of duplicated genes and recognition of their redundant or diverged effects on stress response is currently challenging task in studies of crop plants. In this study we focused our efforts on determination the *ABI1*-related genes functions in *Brassica napus* in drought stress response taking into account its duplicated genom status. In plants, protein phosphatases 2C (PP2Cs) group A form a large gene family, whose many members are a key component and repressor of the abscisic acid (ABA) signal transduction involved in the regulation of multiple signaling pathways through protein dephosphorylation. We identified and characterized 6 *BnaABI1* homeologous genes in the amphidiploid *B. napus* genome. Despite the high sequence homology, these genes exhibit differences in the expression profiles in different tissues, stages of development, in response to the salt stress, exogenous ABA and H_2O_2. To determine whether the *BnaABI1* duplicated genes maintain a functional redundancy or evolved independently for different functions, two *BnaABI1* genes representing the closest and distant orthologs to *A. thaliana AtABI1* gene were characterized functionally. We found that the expression pattern of both *BnaABI1* homologue genes was significantly changed in response to drought stress. To gain a more detailed insight into the *BnaA01.ABI1.a* and *BnaC06.ABI1.b* gene-expression profiles, we studied their promoter activity in transgenic *A. thaliana* plants carrying *BnaA01.ABI1.a* and *BnaC06.ABI1.b* promoters fused to *β*-glucuronidase reporter gene. Both promoters revealed similar activities in leaves, flowers, pollen and in response to exogenous ABA treatment and wounding. However, only one of them was induced under drought stress. Comparative sequence analysis of both *BnaABI1* promoters showed variation in positions of *cis*-acting elements especially important for the ABA- and stress-inducible expression. The combined results has shed new light on diverse role of the *B. napus ABI1* gene family in response to drought conditions. These results provide a framework for understanding the function of *BnaABI1* genes coding protein phosphatases in tolerance of the *B. napus* plants to the environmental stresses.

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Poster 2.2

Complex composition of carrot carotenoid crystals

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Carotenoids belong to important plant secondary metabolites and play many crucial functions in plants including response to abiotic stress. In carrot root, substantial amounts of carotenoids are deposited as crystals in chromoplasts. Previous research indicated that such crystals contain β-carotene but the presence of other carotenoids has remained questioned.
In this work, progeny of a self-pollinated single carrot plant was used and comprised roots characterized by various content and ratio of carotenoids as determined by HPLC: HαHβ - α-carotene (21%), β-carotene (76%), lutein (3%) and LαLβ - α-carotene (5%), β-carotene (77%), lutein (17%). Single carotenoid crystals were identified in carrot root cells using a microscope and were measured directly without compound extraction using a high resolution confocal Raman imaging spectroscopy with the application of a 100x objective.
The shape of the obtained Raman maps corresponded to crystal shapes visible under light microscope. The spectra extracted from various points of the HαHβ map showed a band with a maximum at 1517 cm^{-1} and with a shoulder at 1526 cm^{-1}, characteristic for β-carotene and α-carotene, respectively. The presence of lutein could also slightly contribute to the band shoulder since lutein and α-carotene Raman signals are almost at the same position. Distinguished bands related to both β-carotene and α-carotene/lutein were observed in Raman spectra of the LαLβ root using 488 nm excitation. The spectra shapes were very similar independent on the measurement position within a crystal. This indicates that crystals are composed of more than one compound and that these compounds are homogenously distributed in the whole crystal volume. Thus, despite the fact that the pathway for carotenoid biosynthesis in plants shows different routes of β- and α-carotene conversion from lycopene, both compounds are co-localized and homogenously distributed within a crystal. The question remains open as to whether lutein occurs in such crystals.

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Poster 2.3

Can the fluorescence parameters of German chamomile leaves be predictors of the anthodia yield during water scarcity?

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The resistance of plants to alterations in photosynthetic machinery may be estimated by the analysis of chlorophyll fluorescence (CF). Therefore, the aim of the study was to estimate the impact of soil drought on the photosynthetic apparatus of German chamomile (*Chamomilla recutita* (L.) Rauschert)). As a common weed and medicinal plant, it is considered tolerant to drought. However, its cultivars and strains may be susceptible to water shortage to different extent, and this involves alterations in morphological changes, anthesis and gas exchange. Moreover, some of these genotypes are di- or tetraploid, hence the ploidy level may influence plant response. Considering this, 7-day soil drought followed by 7-day rehydration was applied to plants at the beginning of their generative stage, in a pot experiment. The level of drought was 45% of field water capacity, whereas the control was 60%. Plants of a wild type (WT) and four breeding genotypes, two diploid and two tetraploid were studied. CF parameters were obtained on a dark- and light-adapted leaf using FMS-2 plant fluorometer (Hansatech, UK), whereas the pigment content was measured spectrophotometrically. The fresh weight of the anthodia after the recovery was also analysed.
It was established that WT plants adjust better to water stress than breeding genotypes, because drought resulted in lower dehydration of its leaves, the lowest decrease in the anthodia yield, and the most elastic response of the photosynthetic apparatus. Tetraploid C11/2 strain plants suffered from the highest reduction in anthodia yield (approx. 90%), and it was also the only genotype which revealed nontypical, prolonged alterations in various CF parameters, e.g. a large increase in minimal, maximal, and variable fluorescence of PSII reaction centres in the dark- and light-adapted state, and by the biggest decline in quantum efficiency of PSII electron transport (ΦPSII), photochemical quenching coefficient (qP), and linear electron transport rate (ETR). On the contrary, plants of diploid C6/2 strain revealed the highest photosynthetic potential having the highest constitutive Chl and Car contents, which additionally increased after rehydration, similarly to the values of basic CF parameters. It was accompanied by the relatively high yield of its anthodia. It was concluded that the analysis of the parameters of fluorescence of chlorophyll *a* can be useful in the assessment of the condition of plants during drought, as well as their photosynthetic potential in normal water regime, providing that the analysis of CF parameters is carried out comprehensively. However, the forecasting of chamomile yield on the basis of such analysis is impossible.

Poster 2.4

Detailed analysis of DNA methylome modulation under drought stress in barley reveals differential epigenetic response of leaves and roots and provides a link with drought stress transcriptome

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Adverse environmental conditions have a negative impact on plant growth and crop productivity. One of the strategies determining rapid adaptation of plants in response to environmental stimuli, is the ability to modulate the gene expression by epigenetic mechanisms, such as DNA methylation. In this study, a detailed analysis of barley leaves and roots methylome modulation under drought stress conditions, as well its possible consequences for gene expression, are presented.
The assessment of barley methylome response to water deficiency conditions was carried out on leaf and root samples of plants grown in normal, control conditions, then exposed to severe drought stress, and next after re-watering. Genomic sequences undergoing DNA methylation changes were identified with a novel method, MSAP-Seq (Methylation Sensitive Amplification Polymorphism Sequencing), which involves direct sequencing of regular MSAP amplicons using Next Generation Sequencing methods.
Detailed analyses of about 150,000 loci sequenced per sample revealed that drought stress induced global-wide changes in barley methylome, however both organs differed significantly in stress-induced epigenetic response. Twice as more differentially methylated loci (DMLs) were identified in roots (2.6% of all loci) than in leaves (1%), and the main difference resulted from numerous stress-induced new methylation events occurred in roots. Interestingly, re-watering of analyzed plants resulted in broad reversion of stress-induced epigenetic changes, particularly in leaves, but not in roots, where the addition of water caused another epigenetic response and induced demethylations in new loci. One-fifth of identified DMLs in both roots and leaves represented genic regions, with changes occurring mainly within gene-bodies. Surprisingly, only 2% of genes identified in roots and leaves were common for both organs, however most of them belonged to similar functional groups. Simultaneous transcriptomic analysis carried out with the same experimental material using microarrays, revealed a correlation between differential DNA methylation and expression modulation for numbers of drought-responsive genes. Consequently, a detailed analysis of epigenetic changes induced by stress in roots and leaves, coupled with global transcriptome analysis allowed for identification of the subset of genes which expression upon drought is presumably regulated epigenetically.

This work was supported by the EU-FP7 project no. 289300 "EURoot: Enhancing resource Uptake from Roots under stress in cereal crops"; www.euroot.eu

Poster 2.5

ZPR1 transcription factor is involved in regulation of expression of the circadian-dependent *SsBBX24* gene in *Solanum tuberosum*

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Zinc finger proteins form a relatively large family of transcription regulators in plants. They are important components in the regulation of plant growth and development, and participate also in the responses to stresses. The zinc finger proteins are arranged into several distinct types, based on the number and the location of characteristic residues. Among them, C4-type of zinc finger proteins, that are often described as CX_2-CX_{25}-CX_2-C known as the ZPR1 domain, are characterized by 4 cysteine residues that coordinate zinc ion.
StZPR1 gene was screened from *S. tuberosum* cDNA library by using a yeast one-hybrid system to identify transcription factor that interacts with specific *cis*-regulatory element 'CAACAGCATC' presents in the promoter region of the double B-Box *SsBBX24* gene and controls its expression in the light phase of circadian cycle.
The *StZPR1* cDNA displays an open reading frame of 1512 bp encoding a protein of 504 amino acids with a calculated molecular mass of 54.79 kDa. Computational analysis of StZPR1 protein sequence revealed that it contains the N- and C-terminal regions that form duplicated ZnF module known as the ZPR1 domain. Sequence comparison of the StZPR1 protein from plant species showed that the deduced ZPR1 protein sequence shares 97 % and 73 % similarity with SpZPR1 (from *S. pennelli*) and AtZPR1 (from Arabidopsis).
To confirm validity of StZPR1 interaction with the target 'CAACAGCATC' *cis*-element in the one-hybrid system a cotransformation procedure was applied using the target 'CAACAGCATC' DNA sequence and the mutated sequence. The binding ability of isolated *StZPR1* transcription factor to 'CAACAGCATC' *cis*-element was also verified by Electrophoretic Gel Shift Assay method. Both the *in vivo* and *in vitro* approaches shown that the StZPR1 protein binds the 'CAACAGCATC' *cis*-element.
In order to uncover whether *StZPR1* expression is regulated by the circadian rhythm, the *StZPR1* transcript abundance was investigated in 2-week-old *Solanum* plants entrained in a 14-h photoperiod. As shown no change in *StZPR1* abundance was found during day/night cycle.

Poster 2.6

Flavonoids and other metabolites of fodder grasses involved in tolerance to cold stress

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Acclimation to cold and plant tolerance to freezing conditions is a complex process. Low molecular weight compounds play an important role in protecting plants against freezing conditions. We have acclimated 3 cultivars from 3 fodder grass species from the *Lolium-Festuca* complex. Samples were collected at 3 time points of acclimation and after 3 weeks of regrowth after the freezing test.
We have analyzed changes in profiles of secondary metabolites using ultra-high performance liquid chromatography with UV detector (Waters). Changes in primary metabolites profile were analyzed using gas chromatography (Agilent) combined with mass spectrometer (Waters). Apigenin was used as the internal standard in analysis of phenolic compounds. Adonitol was used as the internal standard for quantitative analysis and C10-C36 alkanes were used as retention indexes in analysis of primary metabolites. Quantities of carbohydrates were analyzed in diluted samples and quantities of other compounds where analyzed in non-diluted samples.
The computational steps for secondary metabolites were performed for the most representative wavelengths (280 nm and 330 nm) and retention times. First, raw data were normalized by mass of samples. The baseline was removed by differentiation. Retention time alignment was done by correlation optimized warping (COW) [1]. Peaks, were detected for individual chromatograms by using profiles of smoothed second derivative. Chromatograms were integrated over peaks. Computations were done in the R system using own scripts. Peaks were annotated to phenolic compounds identified by using mass spectrometer (Bruker) combined with high performance liquid chromatography (Agilent). In *Festuca arundinacea* cv. Kord 51 peaks were found, including 17 unknown compounds. Similarly, 38 and 26 peaks were found in *Lolium perenne* cv. Solen and in *Festulolium* cv. Felopa, respectively and these chromatograms included respectively 12 and 5 unknown compounds.
The TargetSearch program was used to pre-process the GC-MS data by: baseline correction, peak finding, retention time correction and peak annotation according to the Golm Metabolome Database [2]. Suggested identifications were confirmed by comparing them with the recorded mass spectra. Eighty seven compounds were found in the non-diluted samples and 58 in the diluted ones, including 31 and 9 still unknown compounds, respectively.

Acknowledge to National Science Centre for founding project number N310 381839.

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Poster 2.7

Assessment of the genotype-environment interaction in *Lupinus angustifolius* L.

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Ten traditional and unbranched cultivars of narrow-leafed lupin (*Lupinus angustifolius* L.), differentiated in terms of morphological structure and phenological phases, were studied. Field experiments were conducted in 2011-2013 in a randomized complete block design. The number of locations and replications was different in different years of experiences (in 2011: 2 locations and 4 replications, in 2012-2013: 4 locations and 6 replications).
Assessment of the genotype-environment interaction and analysis of seed yield, its stability for various cultivars of narrow-leafed lupin in different environments were the main goal of the research**.**
The results of seed yield were analyzed statistically using the analysis of variance under a linear model and the significance of differences among cultivars was tested using the Fisher F-test. The coefficient of variation was calculated for comparison the differentiation of cultivars in terms of seed yield. The variation components were calculated using the restricted maximum likelihood (REML) method. The assess of the share of each cultivar in the creation of the genotype-environment interaction has enabled by the W_i ecovalence (Wricke 1962). The assess of yield stability was calculated using the Sergen and MStat programs. The regression curves for each cultivar with coefficients of determination R^2 were determined.
The research showed, that seed yield of each cultivar and its stability was different in different environments. Stable genotypes possessed a high ecovalence (low values of W_i = high ecovalence) and their W_i were close to 0. Significant differences in terms of seed yield between cultivars of narrow-leafed lupin were observed. The genotype-environment interaction and the variability between environments were dominant.

Poster 2.8

Changes in the eggplant metabolome upon arthropod herbivore attack

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It is widely accepted that secondary metabolites from different classes are essential for the defence of solanaceous species against many arthropod herbivores. In the case of the eggplant this issue is still unknown. Therefore, it has been verified whether phenolic compounds of the eggplant are, similarly to other Solanaceae, involved in a response to pests commonly colonising this crop under greenhouse conditions and whether the relatively susceptible eggplant cultivar is able to induce/activate this type of response when infested by pests at a low density.
Eggplants (*Solanum melongena* L.), ScorpioF_1 were grown in rockwool slabs fertilized with a solution of nutrients using the drop irrigation system for 12 weeks. Discs (2 cm) were cut out from corresponding leaves of uninfested (control) and thrip- and mite- infested plants at a density of no more than 5-10 specimens/leaf following 1 week of infestation. Three biological replicates consisting of 4 disks each were prepared. Leaf samples were frozen in liquid nitrogen and kept in -80^{o}C until analyses were carried out. Secondary metabolites were extracted with 80 % methanol and their structures were resolved by mass spectrometry
In comparison with the control, in extracts of thrip- and mite-infested eggplant leaves changes in content of hydroxycinnamic acid derivatives, flavonoids and potentially toxic glycoalkaloids were observed. Changes in the content of leaf secondary metabolites triggered by thrips and mite pests suggest that metabolome re-programming took place and imply that even the susceptible eggplant cultivar is able to respond to pests at a low density. Further studies are needed to assess whether the observed metabolomic changes are effective towards arthropod-pest performance.

Poster 2.9

Effect of salinity on carrot (*Daucus carota* L.) seed germination *in vitro*

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Nowadays, salinity of cultivated fields has a devastating impact on the global range. High concentration of salts can cause physiological, morphological and also molecular changes that negatively affect plant productivity and growth. Carrot is classified as a salinity sensitive species and a negative correlation was reported between root yield and salinity above a critical threshold value of 10 mM NaCl.

In this study we compared seed germination potential of five carrot populations (3 breeding lines and 2 landraces) from various origins in salt-stress conditions *in vitro*. Seeds were placed on Murashige and Skoog mineral medium including vitamins and containing 50 – 200 mM NaCl, the ability of germination was assessed four times every 7 days.

In control conditions (no NaCl treatment) 85 – 100% of seeds germinated during 28 days, and 97% of the developed seedlings were morphologically normal. NaCl treatment of 100 mM adversely affected seed germination. Concentrations of 100 – 175 mM delayed germination for all carrot populations and 200 mM NaCl completely inhibited germination of carrot breeding line 2874B. NaCl treatment also restricted seedling development to radicle occurrence and reduced the percentage of morphologically normal seedlings, and these effects were more pronounced if the higher NaCl concentration was used. However, a DLB-A landrace from Iran, showed increased tolerance to NaCl. Up to 80% of normal seedlings developed in the presence of 150 mM NaCl in the medium. These results show that genetic determinants of salt tolerance exists in carrot natural resources that can be potentially exploited for breeding purposes.

Poster 2.10

Arabidopsis MAPKKK18 affects ABA-induced stomatal movement and patterning

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Stomatal development and patterning are regulated by many diverse pathways. Among them, MAP kinase cascade are known as key regulators of guard cells distribution and activity. To analyze the function of MAPKKK18 in these processes, we generated transgenic plants carrying *MKKK18* promoter fused with the GUS reporter gene. We found that *MAPKKK18* promoter is ABA responsive in guard cells. Next, we analyzed if MAPKKK18 is involved in the regulation of stomatal aperture. We found that two distinct *MKKK18* knockout lines increased stomatal aperture under normal growth conditions in comparison to the wild type plants. In response to ABA, guard cells of knockout lines showed strong phenotype and were again significantly more open than in the WT. Interestingly, *MAPKKK18*-overexpressing lines were hypersensitive to ABA-induced stomatal closure. In addition to that, we establish that MAPKKK18 is required for normal stomata development. We compared the number and density of stomata in 6- and 10-day-old seedlings in *MAPKKK18oe*, WT and the *MKKK18* knockout lines. Stomatal density was significantly reduced in both knockout lines, but it was 7% higher in the *MAPKKK18oe* line compared to the WT. Overall, our results suggest that MAPKKK18 is on the top not yet fully established MAP cascade involved in stomatal movement and development.

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Poster 2.11

Dissecting QTLs for responses to water deficit in rye RIL population

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Considering that the morpho-physiological traits that affect the tolerance of crops to drought are quantitatively inherited, the discovery of QTLs should play a crucial role in their improvement through MAS. The increasing number of studies reporting QTLs for drought-related traits and yield in drought-stressed crops (wheat, barley, rice, maize) indicates a growing interest in this approach. Rye is a species known for its excellent tolerance against many biotic and abiotic stresses. Nevertheless, little is known on quantitative loci underlying its resistance to water deficit.
This study was aimed at detecting QTLs that affect changes of some yield-related traits under water-limited conditions. RIL population S120×S76 was used to examine number of spikes per plant (SNPP), number of kernels per spike (KNPS), weight of kernels per spike (KWPS) and mean kernel weight (KW) in two variants of experiment: control plants (well watered) and plants with a reduced water access (50%). The index of drought response was calculated for all examined traits and QTL analysis was carried out using high density, consensus, genetic map of rye. 27 QTLs were identified on all 7 chromosomes, majority of them were these controlling KNPS changes.

Poster 2.12

Accumulation and activity of chloroplast fructose 1,6-bisphosphate aldolase in *Lolium multiflorum/Festuca arundinacea* introgression forms

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Stomatal closure is thought to be one of the earliest responses to water deficit and it is considered to be a main cause of decreasing photosynthetic rate in plants under drought conditions. However, the studies on the non-stomatal drought-induced limitations in CO_2 assimilation rate suggested that the several cell metabolic pathways, including e.g. regeneration phase of the Calvin cycle could be also involved into that process. Our earlier research, performed on *Lolium multiflorum/Festuca arundinacea* introgression forms, showed that the high drought tolerant (HDT) form characterized by higher yield potential during long-term drought selection in the field (14 weeks), had also lower photosynthetic rate after short-term drought treatment in laboratory conditions (11 days in pots), compared to the low drought tolerant (LDT) form, characterized by lower yield potential in the field. Although these two forms significantly reduced stomatal conductance under short-term drought, it was also clearly shown that the level of CO_2 assimilation rate in these conditions was not associated with stomatal aperture. Proteome profiling, based on 2-D protein maps and mass spectrometry protein identification, revealed that the LDT form had higher accumulation level of chloroplast fructose 1,6-bisphosphate (F 1,6-BP) aldolase, which is a key enzyme of the Calvin cycle, and its elevated accumulation level could affect photosynthetic rate. The objective of this study was to compare the differences in chloroplast aldolase accumulation and activity at several time-points of short-term drought in the two previously selected introgression forms. This objective was realized on the basis of: (*i*) analysis of aldolase accumulation level using Western Blot procedure and a specific antibody, and (*ii*) measurements of aldolase activity using the modified method of Sibley-Lehninger. The obtained results showed that a protein accumulation level of chloroplast F 1,6-BP aldolase was higher in the LDT form during drought and in controlled conditions, compared to the HDT form. Aldolase activity also demonstrated higher values for the LDT form at all the analyzed time-points of the experiment. Thus, we hypothesized that higher accumulation level and activity of chloroplast F 1,6-BP aldolase in the LDT form could increase the photosynthetic rate of this genotype.

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Poster 2.13

Morphology and anatomy of the root system of potato cultivars susceptible and tolerant to heat and drought stress

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Potato (*Solanum tuberosum* L.) has specific temperature requirements and develops best at about 20°C. The limits and optimal values for the growth of the above-ground of the potato plant and for the tubers are different – lower for tuberization and tuber growth. Under high-temperature conditions, tuberization is significantly inhibited and photoassimilate partitioning to tubers is greatly reduced. In natural conditions drought and heat stress are two different types of abiotic stresses that occur generally in the field simultaneously. Due to increasing irrigation use on potato plantations and periodic action of heat stress under conditions of good soil moisture, in our current studies the impact of high temperature on potato plants is separated from the impact of drought to identify the potato cultivars less and more tolerant of each of the tested stress. We attempt to identify mechanisms of tolerance to drought and high temperature by means of a number of physiological indicators. Additionally, the studies of morphology and anatomy of root systems have been initiated. The results can be helpful in explaining the problem presented.

The experiment was carried out in 2014 using a device for the production of potato minitubers in aeroponics. The study was conducted on the 17 cultivars: Denar, Lord, Justa, Miłek (very early), Aruba, Bila, Etola, Gwiazda, Hubal, Michalina (early), Etiuda, Finezja, Gandawa, Kuba, Oberon, Stasia i Tetyda (medium early). Minitubers were planted in baskets at the end of April. The root system has developed freely in the space of aeroponic chamber. The liquidation of the plants was carried out during the flowering period, at the beginning of tuberization.The developmental state of the above-ground parts of the plant as well as the length, the fresh and dry weight of root systems were determined. Samples of basal parts of the oldest roots were also taken for anatomical examination. Hand-made root cross-sections were observed using bright field and UV fluorescence optics. Digital images were taken for anatomical description and morphometric analysis.

Comparison of potato tolerance to drought and high temperature conditions and the morphology and anatomy of the root system will be carried out after completion of all the tests.

Poster 2.14

The development and evaluation of a plant phenotyping system for the assessment of root system growth and architecture

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The established system for the phenotypic analysis of plant root traits presented below enables the precise characterization of root system growth dynamics and architecture of the monocotyledonous plants during their growth. Due to the application of automatic drip-line irrigation system for the supplementation of plants with medium, the precisely controlled plant growth conditions can be obtained for the short-term experiments. The system enables effortless, accurate and highly repeatable analysis of the root system features for cereal plants.

The designed system employs a controlled drip irrigation line, controlled remotely by the programmable logic controller (PLC). The use of PLC adapter facilitates the automated control of all system modules (water pumps, air pumps, heating devices) allowing the adjustment of the rate of medium flow for the supplementation of plants. The system allows the application of measuring sensors for the constant monitoring of culture medium parameters: temperature, pH, redox state, concentration of specific ions. PLC controlled experiment can be monitored and modified by setting the threshold values of desired medium parameters. The permanent sensing of desired culture medium parameters can be highly useful for mineral nutrition studies and abiotic stresses response testing. Installed drip-lines are injected onto transparent acrylic tubes which are placed in opaque cover tubes to protect the roots from the light. That allows an intravital observation of the growth of the root system of plants, with the application of a transparent substrate - soda-lime glass beads. Acrylic tubes are provided with a bottom opening to ensure proper draining of the medium. The use of soda-lime glass beads in the conducted experiment allows also for the improvement of root system cleaning process which affects the final image quality. Enhanced imaging quality contributes to the increase in the precision of the results obtained in course of the analysis of root parameters using specialized root scanners coupled with the WinRHIZO system (Regent Instruments Inc.). The parameters generated with the use of the system include: total length of the root system [cm], root system surface [cm^2], root system volume [cm^3] and root diameter [mm].

The developed system enables a non-invasive root system growth observation adjusted for subsequent root image acquisition with a reduced background noise. The method combines automated control of plant growth conditions with a good imaging quality and high growth parameters replicability.

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Poster 2.15

Arabidopsis MAPKKK18 interacts with protein phosphatase 2C ABI1 and is regulated by the ubiquitin proteasome pathway

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The phytohormone ABA recruits many diverse elements for the generation and transmission of endogenous signals. Among them protein kinases and protein phosphatases are key proteins involved in ABA signal transduction. Nevertheless, little is known about the role of MAPKKK in this pathway. Available data demonstrate that ABA leads to changes in *MAPKKK18* gene expression. Therefore, to analyze whether MAPKKK18 is involved ABA pathway, we tested if MAPKKK18 kinase activity is regulated by ABA. We found that MAPKKK18 is indeed ABA responsive. Because our previous results showed that MKKK18 expression was significantly affected in the *ABI1* knockout, we analyzed the interaction between MAPKKK18 and ABI1 using yeast two-hybrid, pull-down and BiFC assays. We found that MAPKKK18 interacts with ABI1 PP2C. Then using *in vitro* and in-gel assays we analyzed the effect of ABI1 on MAPKKK18 activation. We found that ABI1 inhibits MAPKKK18 activity. Consequently, we also examined the turnover of MAPKKK18 using cell-free degradation assay. These results showed that MAPKKK18 is degraded by the proteasome pathway. Notably, the MAPKKK18 degradation was delayed in the *ABI1* knockout line. All this data indicate, that MAPKKK18 is regulated both ABI1 PP2C and the proteasome pathway.

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Session 3:
Plant-microorganism interactions

Identification, quantification and characterization of selected oilseed rape pathogens in air samples

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Fungal propagules dispersed by wind and wind-driven rain can be readily monitored when captured on rotating tapes of volumetric spore traps. The SPEC project (www.spec.edu.pl) has operated Burkard and Lanzoni spore traps situated near winter oilseed rape fields in 10 climatic zones of Poland, since 2004, for seasonal monitoring of spore concentrations in air particles. A similar monitoring system operates, at a smaller scale, in Harpenden, UK, at Rothamsted Research. Tapes from the traps are routinely processed for spore counts followed by DNA extraction, which enables the determination of spores of *Leptosphaeria maculans* and *L. biglobosa,* associated with the phoma stem canker disease by both endpoint and quantitative Real-Time PCR approaches. Additionally, it is becoming increasingly possible to evaluate these DNA samples for avirulence allele variants and for monitoring molecular changes in fungicide targets within pathogen populations. Primers designed for the detection of the virulence alleles *avrLm1* and *avrLm6* as well as for *erg11*, encoding the membrane-bound sterol biosynthetic enzyme ergosterol demethylase (= CYP51) from *L. maculans* were used to characterize propagules captured on tapes. There were differences in sensitivity among the three qPCR approaches used for quantification of the captured fungal material. Dual-labeled fluorescent probes proved to be more efficient than the SYBR Green-based primers. For both countries, the *avrLm1* and *avrLm6* were easily detected in spore populations thereby enabling the characterization of aerial *L. maculans* propagules at the race level. Quantitative Real-Time PCR is a dependable and increasingly affordable means for enabling precise measurement of DNA extracted from spores captured in air samples and different methodological approaches exist to facilitate choice flexibility. Spore concentrations on tapes can be easily related to DNA yield to enable a comparison of sensitivities of the quantification methods employed. Pathogen's DNA from tapes can be characterized using molecular markers for such traits as virulence, fungicide resistance and pathogenicity.

Fusarium community on wheat grain samples originating from different regions of Poland in 2013

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Fungi from the genus *Fusarium* cause Fusarium head blight (FHB) of wheat (*Triticum* sp.). The disease may cause considerable losses of grain yield. Moreover, *Fusarium* spp. produces heat-resistant toxins, which can lead to serious animal and human health disorders, if present in food and feed. The aim of this work was to evaluate the level of grain infestation by *Fusarium* spp., the species composition and the resistance of different wheat cultivars to FHB. Four cultivars of wheat were sprayed with fungicides at flowering time and the efficacy of this treatment was investigated. The experiment was done in 2013, in four regions of Poland with different weather conditions. Seeds of four wheat cultivars of different origin and level of susceptibility to FHB were tested using ISTA-based seed health test on PDA medium. Preliminary identification of *Fusarium* species was done with morphological characters of cultures on PDA and SNA media. Species identification was done using CAPS, dCAPS and PCR-RFLP molecular methods. The analysis was based on 570 isolates, varying from 73 to 271 individuals per site.
The level of wheat grain infestation varied between locations, wheat cultivars and the use of fungicide application. The most prevailing species were *F. graminearum* and *F. poae*, whereas *F. avenaceum* and *F. culmorum* occurred less frequently. Other species, such as: *F. tricinctum*, *F. equiseti*, *F. venenatum*, *F. cerealis* and *F. sporotrichioides* were found sporadically. The highest percentage of FHB-infested grains was found in the region of Carpatian Foothills (south-east of Poland), while the lowest in Great Poland (central-west). The most resistant wheat cultivar was 'Arina' (Agroscope, DSP, Switzerland), while 'Bogatka' (DANKO, Poland) was the most susceptible one. Two other cultivars 'Tonacja' (HR Strzelce, Poland) and 'Michigan Amber' (Michigan State University, USA) were infected by *Fusarium* spp. at intermediate levels. The application of the fungicide decreased *Fusarium* spp. occurrence on grains by approximately 20%. Molecular tools allowed to identify properly numerous isolates of *Fusarium* species complex in a short time and can be proposed as a fast method of characterization of the main components of FHB causal agents.

Sources of genetic resistance to *Plasmodiophora brassicae*

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Plasmodiophora brassicae is a pathogen of Brassicas, causing considerable yield loss both to vegetables, such as: cabbage, cauliflower, broccoli and swede and to agricultural crops, such as: oilseed rape, turnip rape or various kinds of mustard. Moreover, the pathogen can be carried over to new crops or revived by cruciferous weeds, that are growing on most of the fields. The microorganism is classified as a protist belonging to the supergroup of rhizaria, similarly to protists attacking potatoes (powdery scab), beetroot (rhizomania) and watercress (crook root). In contrast to some other plasmodiophorids, such as *Polymyxa graminis* attacking barley, it does not transmit any viruses. Nevertheless, the pathogen is very damaging, due to complete transformation of roots into galls (clubroot), what may lead directly to plant death, especially in case of longer water deficiency in soil. Integrated control of clubroot includes the increase of soil pH, crop rotation with decreased use of oilseed rape, weed and volunteer control, the use of clean soil machinery, chemical decontamination of soil, and – first of all – the use of resistant or tolerant cultivars. Fortunately, there are several possible sources of resistance genes that can be incorporated into Brassica crops during breeding practices, with some available in *Brassica napus*, and the others originating from *B. rapa* or *B. oleracea*, as well as *Raphanus sativus*. The synthetic *B. napus*, combining the resistance from *B. rapa* ECD-04 and *B. oleracea* cv. Verheul is currently the source of the first cultivar of oilseed rape resistant to clubroot ('Mendel'), with one dominant, race-specific resistance gene. The cultivar was first approved in 2000 in the UK and one year later in Germany. Most of current cultivars of oilseed rape resistant to *P. brassicae* carry resistance genes from the same source. There are, however, several other genes that can be explored and combined, some of them closely linked, bringing resistance to a few races, and some race-specific. The choice of the best resistance sources requires knowledge about the population of the pathogen. Therefore, our studies include the evaluation of races of *P. brassicae* in different areas of Poland. The search concerns oilseed rape plants and cruciferous weeds encountered in fields infected by *P. brassicae*. According to widely used system developed by Somé et al. (1996) we have found 4 races of the pathogen, with the prevalence of P1 and P3. However, we propose an expansion of the standard set of genotypes by the addition of cv. 'Mendel' or one of cultivars bearing the same resistance genes, to further subdivide the pathotypes of *P. brassicae*.

Antagonistic potential of *Trichoderma* against pathogenic *Fusarium* species – production of volatile antifungal metabolites

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The aim of this study was to examine the ability of seventy-seven isolates belonging to eight different *Trichoderma* species to form 6-n-pentyl-2H-pyran-2-one and other volatiles using solid phase microextraction (SPME) coupled to gas chromatography-mass spectrometry (GC-MS). In addition, the inhibitory effect of 6-PAP on six toxigenic *Fusarium* species, considered to be the most important plant pathogens worldwide, was studied.
The presence and the concentration of 6-PAP in cultures of *Trichoderma* isolates varied significantly. This characteristic appeared to be isolate-specific, and not species-specific. 6-PAP was detected in the headspace of all *T. atroviride* and *T. viridescens* isolates, as well as in individual cultures of *T. citrinoviride* (4 isolates), *T. hamatum* (8 isolates), *T. viride* (3 isolates). No 6-PAP emission was observed in cultures of *T. koningii*, *T. harzianum* and *T. virens* isolates. Furthermore, the antagonistic potential of purified 6-PAP towards *F. cerealis, F. proliferatum* and *F. subglutinans* isolates was tested during the study. The addition of 40 µg/plug of 6-PAP caused a 100% inhibition of the growth of all targeted *Fusarium* isolates from 5 to 18 days of incubation.
Apart from 6-PAP, seventy-seven *Trichoderma* isolates investigated in this study produced various number and amounts of volatile compounds. Using SPME extraction methods, it was possible to identify over forty volatile metabolites in the headspace of their cultures on PDA. Among them, the most commonly produced metabolites were 1-octene-3-ol, isoamyl alcohol, 3-octanone, cyclohept-3-en-1-one, 2-pentylfuran, linalol isobutyrate, toluene, D-limonene, α-bergamotene. Seventeen of the detected compounds have never been reported previously as a secondary metabolites of *Trichoderma*.

Poster 3.2

Identification and characterization of bacterial strains isolated from mature compost obtained from plant debris

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Microbial vaccines containing selected strains of bacteria have an effect on chlorophyll content, flowering and rooting of plants and contributes to the biological protection against the penetration of roots by soil-borne pathogens. Moreover, plants can easier absorb water and selected nutrients. Vaccines beneficially affect the microbiological quality of a substrate for plant growth. They mostly consist of microorganisms isolated from the rhizosphere or composts prepared on the basis of plant debris. Microorganisms isolated from the compost usually have proteolytic, cellulolytic and amylolytic properties. They usually originate from the genera: *Bacillus, Clostridium, Pseudomonas, Bacteroides* and *Ruminococcus*. The aim of the study was the isolation of bacteria from the mature compost made on the basis of sewage sludge and plant debris, and the identification and characterization of the metabolic properties of strains present in this medium. Based on the initial identification and analysis of the biochemical properties some bacterial strains have been selected for further analyses. The goal of this work was to obtain candidate isolates for the creation of microbiological vaccine and to study its usefulness for the optimization of cultivation of the most popular bedding plants such as geranium, sage and marigold. Isolation of bacteria from the mature compost (50% sludge + 20% of wheat straw +30% sawdust) was performed on a standard agar medium (Merck). Isolated bacteria (70 colonies) were propagated on liquid medium and identified based on the ITS sequences analysis. Subsequently, various biochemical properties were determined. Most of the isolates belonged to Gram-positive bacteria. Gram-negative bacteria were observed much less frequently. With the exception of two strains, all of them produced catalase. Most of the strains showed amylolytic and proteolytic properties with various activity. All isolates showed cellulolytic properties, some bacteria did not form urease. Strains selected on the basis of biochemical activities (50 isolates) were identified based on ITS sequencing and restriction digestion patterns. Over 56% of the species belonged to *Bacillus amyloliquefaciens,* 23% were identified as *B. subtilis* and 4% belonged to *B. firmus*. The remaining 18% consisted of such genera as *Actinobacter, Brevundiamonas, Pantotea, Stenotrophomonas* and *Microbacterium*.

The study was conducted within the project NCN no. N N305 036140.

Poster 3.3

The detection of *Plasmodiophora brassicae* from plant roots and soil samples using Loop-mediated isothermal amplification

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Plasmodiophora brassicae, the cause of clubroot, is a very serious problem, preventing from successful and profitable cultivation of oilseed rape in Poland. The pathogen was found in all main growing areas of vegetable brassicas and oilseed rape. The aim of this work was to elaborate a fast, cheap and reliable screening method to detect the amount of the pathogen in samples of oilseed rape plants and soils. To achieve this aim the Loop-mediated isothermal amplification (LAMP) method has been chosen. The set of 3 primer pairs was elaborated using LAMP primer designing software. The detection was performed with the GspSSD polymerase, isolated from bacteria *Geobacillus* sp., with strand displacement activity. DNA extraction from clubbed roots and soil obtained from field infected by *Plasmodiophora brassicae* was done using Novabeads Plant DNA Purification Kit containing magnetic beads. The reaction mix was incubated at 64^{o}C for 60 min and then heated at 85^{o}C for 2 min to terminate the reaction. The visual detection was done using Genie II Ultra Rapid Gene Amplificator (Novazym Polska). The performance of the LAMP test was compared to Real-time PCR method with TaqMan chemistry, which proved its usefulness and higher resolution than the biotest with the use of bait plants. The detection with LAMP proved its usefulness both in case of plant and soil samples. Moreover – it was easier, faster and independent from the age of plant tissues and soil pH.

Poster 3.4

Cellulases as an versatile factors in interactions fungi with plants: cellulolytic enzymes as an infectious agent of *Fusarium* pathogens and as an inducer of plant resistance by the *Trichoderma* fungi

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Complex of cellulolytic enzymes is present both in the fungi of the *Fusarium* genus, which are frequent pathogens of many plants, including important crop species, as well as in the fungi of the *Trichoderma* genus, which, in turn, show a potential as Biological Control Agents in biological plant protection. The activity of the cellulases from *Fusarium* fungi slightly etch plant tissues, thereby allowing the pathogen to penetrate the interior of the plant and promoting the infection process. The role of cellulose action is different in fungi of the *Trichoderma* genus, in which the cellulolytic enzymes catalyze the hydrolysis of cellulose, *e.g.* in the root zone of plants, allowing the fungi to penetrate the plant tissue. *Trichoderma* presence increases the systemic immune response of the plant. Activities of the cellulolytic enzyme complexes from fungal isolates belonging both to *Fusarium* and *Trichoderma* genera were measured in a standard laboratory test, deploying a spectrophotometric method - Filter Paper Assay (FPA) with the use of 3,5-dinitrosalicylic acid (DNS). The absorbance was measured at wavelength λ=530 nm. Most of *Trichoderma* isolates exhibited higher cellulolytic activities than *Fusarium* isolates. The highest cellulolytic activity was measured for the AN 836 isolate of *T. harzianum*. Two isolates of *Fusarium*: *F. acuminatum* and *F. culmorum* displayed higher activity than control mutant of *T. reesei*.
Dedicated primer sets were designed to amplify partial sequences of cellulase-coding genes from the strains studied. PCR-amplified marker fragments were sequenced. A phylogenetic tree was calculated based on the sequence of the gene fragment encoding the endoglucanase V (*eg5*) from *T. reesei* and with the use of the sequences of its homologues obtained from 14 strains of *Fusarium* and 15 strains of *Trichoderma* genera. An evolutionary comparison has been made between the fungi from genus *Fusarium* and *Trichoderma*. Considerable level of DNA sequence divergence was found between both genera. Moreover, numerous inter-specific polymorphisms were detected allowing to distinguish individual species, which makes the studied fragment another good candidate for phylogenetic marker to be used in the evolutionary studies of diverse *Hypocreales* fungi.

Poster 3.5

Identification of the pea pathogenicity (*PEP*) cluster sequences in genomes of different *Fusarium* spp. isolated from pea seeds

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Legume crops, including pea (*Pisum sativum* L.), are exposed to a range of fungal infections. The contamination of pea seeds with fungi and mycotoxins causes economic losses for worldwide production. However, legumes produce a diverse secondary metabolites playing a role of the defense agents against fungi but also acting as signal compounds. Different biochemical pathways are involved in plant response to infestation and one of them is the ability of the pea plants to produce the antimicrobial compound – pisatin. It was the first chemically identified phytoalexin produced by the pea plants at the early stages of defense response.
Three *Fusarium* species (*F. oxysporum*, *F. solani* and *F. avenaceum*) are known to be pea pathogens, capable of causing the changes in composition of enzymes active in infected pea plants as well as the degradation of some isoflavones.
Fusarium oxysporum and *Haematonectria haematococca* (*F. oxysporum*) are able to transform pisatin into a non-toxic compound. Detoxification of pisatin is catalyzed by pisatin demythelase (pda). Both pathogens contain a cluster of genes known as the pea pathogenicity (*PEP*) cluster and one of the cluster genes is the pisatin demythelase (*PDA*) gene. *PEP* cluster of *Haematonectria haematococca* contains six genes: *PDA1*, *PEP1*, *PEP2*, cDNA3, cDNA4 and *PEP5*. Four genes in this cluster are responsible for virulence against pea (*PDA1*, *PEP1*, *PEP2* and *PEP5*). Nine *PDA* genes have been identified in *Haematonectria haematococca* and four of them have been sequenced: *PDAT9*, *PDA6-1*, *PDA4* and *PDA1-1*.
The main aim of the research was to identify the *PDA* sequences in genomes of different *Fusarium* strains isolated from pea seeds collected in 2011-2013 seasons. Fungal isolates were identified based on the analysis of the translation elongation factor (*tef*-1α) gene partial sequence. Sequences of *tef*-1α and *PDA* genes were compared to the NCBI GenBank-deposited sequences and aligned with ClustalW algorithm. To show the divergence of the *PDA* sequence among the strains, phylogenetic relationships were reconstructed using Maximum Parsimony approach.

Session 4:
Biotechnology in basic and applied research

Extending the oilseed rape gene pool with resynthesis *Brassica napus* L.

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Brassica crop species have become one of the world wide most important source of vegetable and vegetable oils. The development of plants from *Brassica* ssp. was accomplished by substantial progress in breeding and biotechnology. *Brassica napus* (2n=38, genome AACC) is a natural amphidiploid that originated from several independent spontaneous hybridizations between the diploid species *B. rapa* (2n=20, genome AA) and *B. oleracea* (2n=18, genome CC). Intensive quality breeding combined with the limited geographic range of *B. napus* has led to a narrow genetic basis in this species. In contrast, the both progenitors are highly polymorphic and therefore offer a broad genetic variability that can be exploited for oilseed rape improvement by the use of resynthesis (wide hybridization). Developing resynthetic *Brassica napus* lines has provided important basic germplasm for further improvements of seed yield (namely by effect of heterosis) and seed quality traits as well as disease and pest resistance.

A major problem encountered with the use resynthesized lines (RS) of *B. napus* in hybrid breeding is the quality of seed oil (high levels of erucic acid) and seed meal (high glucosinolate content), which do not comply with double-low quality oilseed rape**.** Additional breeding treatments are needed before the introduction of resynthesized *B. napus* to practice.

The presentation will reveal the results of research on introduction of resynthesized *Brassica napus* germplasm to actual breeding lines, by creating of semi-RS with double-low quality as well as a genetically distant from current natural oilseed rape.

In this study, resynthesized oilseed rape was obtained through crosses between *B. rapa* ssp. *chinensis var. chinensis* (pak choy) and *B. oleracea* ssp. *acephala* var. *sabellica* (curly kale) using the embryo rescue technique. Double-low winter oilseed rape lines having the *Rfo* gene for CMS *ogura* were crossed with two resynthesized oilseed rape lines. Populations of large numbers of doubled haploids, (DH), were developed from F1 hybrids semi-RS (semi-resynthesized oilseed rape) by the use of the microspore *in vitro* culture method. The seeds of the obtained DH lines were analyzed biochemically with regard to the double-low quality (zero erucic acid and low glucosinolate content). Among the populations of the, DH genotypes with 00-quality and with the *Rfo* gene were selected.

The large genetic distance between selected semi-RS DH lines *B. napus* and existing natural lines of winter double-low oilseed rape has been consequence of the introgression of resynthesized germplasm to current winter double-low oils.

The use of in vitro embryo rescue cultures for introduction of resistance genes from related *Brassica* species into oilseed rape (*Brassica napus* L.)

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The data resulting from our own research, as well as those available in the research literature shows, that an important problem to be solved is the resistance of rape (*Brassica napus)* to disease, especially blackleg (*Leptosphaeria maculans*) and powdery mildew (*Peronospora parasitica*). Also, in recent times, due to the prohibition on the use of certain pesticides in the EU emerged the problem of insect resistance, especially on cabbage root fly (*Delia radicum*), aphids (*Brevicoryne brassicae)* and flea beetle (*Phyllotreta cruciferae*). Among species of *Brassica* genus, there are those who have resistance to the above-mentioned diseases and pests, and can thus be used to convey these features to rape. Wild species of the genus *Brassica,* which show resistance to the above-mentioned pests are, for example, *B. tournefortii* and *B. fruticulosa* , and the other species that are used in the interspecific crosses conducted in the Department of Genetics and Plant Breeding are those having such genes as resistance to powdery mildew (*S. alba, R . sativus, B. campestris* ssp. *pekinensis* and *rapifera*) or resistance to blackleg (*B. nigra, B. juncea, B. carinata, Sinapis arvensis*).

By the year 2014, we performed interspecific crosses, in which the maternal forms were selected rape cultivars and male-sterile line MS-8 and as pollinators were used the following species: *B. tournefortii* , *B. fruticulosa, S. alba, R. sativus, B. campestris* ssp. *pekinensis*.and ssp. *rapifera, B. carinata* and *B. juncea.* All hybridization were performed with the application of an in vitro culture of isolated embryos according to the method discrabed by Wojciechowski (!985, 1998). The immature embryos were isolated from young silique at different developmental stages i.e. heart and early and late torpedo, 14-19 days after pollination. The effectiveness of interspecific crosses varied widely depending on which species where used as pollinator. The lowest efficiency was observed in the combination in which the pollinator were *B. tournefortii* or *B. fruticulosa.* In this case, there were no seeds set on the plant and the efficiency measured by the number of hybrid regenerated from cultured embryos was less than 1%. In other combinations efficiency ranged from 2.8% to 41,3%.

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Development of tobacco doubled haploids resistant to *Tomato spotted wilt virus* and *Thielaviopsis basicola* using biological assays and SCAR markers

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Black root rot (BRR) and tomato spotted wilt virus disease are the most economically important problems in tobacco. BRR is caused by soil-born pathogen *Thielaviopsis basicola*. The fungus infects roots, which results in reduced yield and lower leaves quality. Tomato spotted wilt virus (TSWV) occurs worldwide and causes serious losses of tobacco. The most effective way to minimize damages caused by pathogens in tobacco would be develop varieties with resistance to multiple diseases. Intervarietal transfer of resistance using classical breeding takes several years to accomplish. An alternative can be provided by regeneration of doubled haploid via anther culture and stem *in vitro* culture. Greenhouse screening techniques and SCAR markers technology identifying resistant plants decrease a number of haploids that need to be carried through successive generation. The aim of this study was to develop doubled haploid combining resistance to *T. basicola* and TSWV as well as possessing satisfactory morphological characteristics. Cultivar Wigola carrying BRR resistance derived from *N. glauca* and newly developed breeding line PW-834, which resistance to TSWV is tightly associated with deformed plant morphology, were crossed. The anther culture technique and flow cytometry analysis were used to produce haploids of F_1 hybrids. Anther-derived haploids were vegetatively propagated and clones were simultaneously screened for BRR and TSWV resistance. Plants were exposed to *T. basicola* after transplanting to peat mix inoculated with the pathogen. After four weeks roots were scored by microscopic evaluation. Virus inoculation tests were performed in greenhouse upon mechanical inoculation with TSWV. The plants were examined for the presence of the virus using DAS-ELISA. The microscopic evaluation of roots showed that 54.5% of haploids had no symptoms of *T. basicola* and were regarded as resistant. In turn, the frequency of TSWV symptomless and ELISA negative plants accounted for 12.4% of the total. The segregation of TSWV resistance gene deviated substantially from the 1:1 expected ratio. SCAR marker-assisted analysis of TSWV resistance rejected the hypothesis that the discrepancies in segregation pattern were attributed to irregular expression of TSWV resistance gene. Stem pith fragments from twenty four haploids combining resistance to *T. basicola* and TSWV were cultured and fifteen doubled haploids DH (R_0 generation) were obtained. DH of R_1 generation expressed the same degree of resistance as initial haploids. Doubled haploids of R_1 generation were also evaluated in terms of agronomic traits.

Freeze-drying of plant tissue containing HBV surface antigen for the oral vaccine against hepatitis B

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The global numbers of Hepatitis B Virus (HBV) infections, chronic carriers and related post-disease morbidity and mortality, particularly in developing countries, have been steadily growing. Freeze-dried formulations are attractive regarding priorities of efficacious, cost-effective, and reliable mass hepatitis B vaccination programmes in developing countries. Initial experiments provided positive results regarding immunisation with freeze-dried material containing the small surface antigen of HBV (S-HBsAg). Nevertheless, lyophilisation of plant material required further investigation since 90% degradation of S-HBsAg was observed during that process . The aim of this study was to develop a freeze-drying protocol facilitating successful processing of plant material containing the S-HBsAg while preserving its VLP structure and immunogenicity. Freeze-drying of the antigen in lettuce leaf tissue, without any isolation or purification step, was investigated. Each process step was consecutively evaluated and the best parameters were applied. Several drying profiles and excipients were tested. The profile of 20ºC for 20 h for primary and 22ºC for 2 h for secondary drying as well as sucrose expressed efficient stabilisation of S-HBsAg during freeze-drying. Freezing rate and post-process residual moisture were also analysed as important factors affecting S-HBsAg preservation. The process was reproducible and provided a product with VLP content up to 200 µg/g DW. Assays for VLPs and total antigen together with animal immunisation trials confirmed preservation of antigenicity and immunogenicity of S-HBsAg in freeze-dried powder. Long-term stability tests revealed that the stored freeze-dried product was stable at 4°C for one year, but degraded at elevated temperatures. As a result, a basis for an efficient freeze-drying process has been established and a suitable semi-product for oral plant-derived vaccine against HBV was obtained.

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Oral presentation

Characterisation of HMW glutenin subunits in common wheat by cIEF

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Capillary isoelectric focusing (cIEF) based on differences in isoelectric points (pI) is a powerful electrophoretic technique used for protein separation and characterization, determination of the isoelectric point (pI) of proteins and analysis of charge heterogeneity of proteins. The aim of this work was to evaluate two-step cIEF technique for the characterization of charge heterogeneity of high molecular-weight glutenin subunits (HMW-GS) from bread wheat grains. With regard on specific properties of HMW-GS, which are indissoluble in water and salts and have tendencies to aggregation and precipitation separations were performed with different concentration of urea, ampholytes, detergent (lauryl sulfobetaine), and other IEF-specific optimizations. In this study, methods using neutral and PVA-coated capillaries were developed. Particularly good reproducibility and well-resolved charge isoform profiles were obtained by introducing a mixture of carrier ampholytes (pH 3-10 and pH 5-8), a high concentration of urea and SB3-12 as detergent in a sample solution during separation. One major and one or two minor isoforms were observed for the individual HMW-GS. These isoforms were satisfactorily separated using a pH gradient into two groups: y-type isoforms and x-type isoforms encoded by the *Glu-B1* locus with shorter migration times and remaining x-type isoforms with longer times. The presented method produced from nine to eleven isoforms for all wheat HMW-GS with p*I* points in the range of 4.7 to 7.0. Generally, the minor isoforms were more acidic compared with the major isoform. The y-type subunits had an approximately neutral character (p*I* 6.7-7.0); however, x-types showed a weakly acidic character (p*I* 4.7-5.2), with the exception of subunits encoded by the *Glu-B1* locus. The obtained cIEF profiles were compared with CE electropherograms. Generally, the number of detected isoforms for the particular HMW-GS detected using both methods was similar. After once first according to authors' knowledge exactly pI of major and minor x-type and y-type HMW-GS are determined.

Oral presentation

Tissue-specific expression analysis of cytokinin dehydrogenase genes in common wheat

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Approximately 200 various active particles are currently classified as cytokinins i.e. phytohormones participated *inter alia* in plants growth and development. Cytokinin dehydrogenase (CKX) is one of the enzymes involved in regulation of cytokinin metabolism. Its uniqueness relies on the ability to conduct an irreversible reaction of cytokinin decomposition. *CKX*-encoding genes occur in groups known as families and are composed of various number of genes. Over the last three years, ten of them have been identified in wheat and the same number in barley. The biological role of individual *TaCKX* genes in wheat remains unknown. The main aim of our study is detailed analysis of biological function of particular *TaCKX* genes. We hypothesized that quantitative expression profiles of individual *TaCKX* genes may indicated their role in wheat. The hypothesis was partially supported in our previous study on barley, and in wheat it requires analysis of *TaCKX* expression in wild-type plants, obtaining modified wheat lines with reduced expression of particular *TaCKX* and thorough characterization of these lines. In this study we profiled the expression pattern of each known *TaCKX* gene in various tissues and developmental stages of three spring wheat cultivars i.e. Torka, Brawura and Ostka Smolicka. The level of *TaCKX* expression was analysed in seven tissues i.e. seedling roots, young leaves, two developmental stages of immature inflorescences and spikes at 0, 7 and 14 days after pollination (DAP). The quantitative Real-Time PCR showed that the majority of *TaCKX* genes are expressed at relatively low levels in comparison to the reference gene, which was a gene encoding cell division control protein of AAA-superfamily of ATPases. Only *TaCKX2* expression in 7 DAP spikes reached the level comparable to the one obtained for reference gene. The comparison of particular gene expression levels in the set of tissues showed that four of them i.e. *TaCKX1*, *TaCKX2*, *TaCKX10* and *TaCKX11* were expressed predominantly or uniquely in developing spikes. *TaCKX3* showed higher level of expression in 7 DAP spikes and leaves in comparison to other tissues, while *TaCKX6* in roots. *TaCKX4* was mainly expressed in leaves but lower levels of its expression were observed also in immature inflorescences and 0DAP spikes. The expression of *TaCKX8* was noted merely in roots and *TaCKX9* in roots and immature inflorescences. In summary, it could be concluded that the expression level of each *TaCKX* gene is tissue-dependent, while expression profiles show gene-specificity.

Poster 4.1

Characterisation of the level of crossability during wide crosses within *Triticeae*

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One of the major goals in plant breeding is to improve crop cultivars and get new cultivars with desirable traits. In plant breeding programs new traits resources are sought among wild relatives.
Triticale (X *Triticosecale* Wittmack) is synthetically received genus, therefore, there is no wild relative. This crop combines quality and yield of wheat and resistance of rye. The level of its genetic diversity is low. Increasing of biodiversity is possible using wide crosses with wild species (*Aegilops sp.*) or wheat and rye.
In presented research 20 cross combination of primary triticale was obtained. The highest wheat crossability (8.6 %) was observed for cultivar Matraderecskei and for rye it was 998 (Amilo x *Dasypyrum villosum*) line (3.1 %). There were also obtained 11 combinations of intergeneric hybrids between few species from *Aegilops* (*Ae. crassa*, *Ae. geniculata*, *Ae. longissima*, *Ae. perigrina*) and different common wheat and triticale genotypes. Accordingly, we received 14 intergeneric hybrids and the estimated total crossability reached 6.3 %.

Presented research are carried out within the framework of project "Development of novel triticale genetic sources on the basis of wide crosses" funded by Polish Ministry of Agriculture and Rural Development.

Poster 4.2

Identification of the *Rht-D1a* and *Rht-D1b* alleles in winter wheat cultivars

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One of the most important agronomic traits of cereals is their resistance to lodging, which can be limited by reducing the height of the plants by introducing semidwarfing genes to cultivars. For this purpose *Rht-D1b* allele, resulting from a point mutation wild form of the gene *Rht-D1a*, is one of the most commonly used semidwarfing gene. Its source is the Japanese variety Norin 10. The presence of both alleles can be stated only by specific markers located on chromosome 4DS. The aim of the study was to verify the effectiveness of specific markers linked to *Rht-D1a* and *Rht-D1b* allels and analysis of 22 winter wheat cultivars in terms of its presence. The plant material designed to verify the effectiveness of the markers were: old high polish cultivars (Ostka Grubokłosa, Leszczyńska, Dankowska Graniatka, Biała Kaszubska), reference cultivars with allele *Rht-D1b* (Wa7643, Wa7644, Wa7645) obtained from the National Small Grain Collection, United States Department of Agriculture, Agricultural Research Service Aberdeen - Idaho United States and semidwarfing varieties from the wheat collection from our Department (CWW 90/3, Heven, GA1b47, PBIS 98/85). The presence of *Rht-D1b* allele was confirm in references cultivars and semidwarfing varieties: CWW 90/3, GA1b47, PBIS 98/85. In all the other tested varieties *Rht-D1a* allele were occurred. Identification of the marker DF-2-WR2 of *Rht-D1a* gene and DF-MR2 of *Rht-D1b* gene was carried out on 22 winter wheat cultivars of different origin. The PCR reactions were conducted according to the protocol of Ellis et al. (2002). Two pairs of primers were used in the study. One generated a 264bp product characteristic allele *Rht-D1a* and present in plants of normal height; the latter generated a 254bp band characteristic *Rht-D1b* allele occurring in semidwarfing genotypes (*Rht-D1b* allele). The marker of *Rht-D1b* allele was found in 5 of 22 studied genotypes. They were cultivars: CWW90/3, Atlas, Rosario, Muszelka, Genou. In all the other varieties *Rht-D1a* allele were identified.

The research is carried out in the project NCBR - PBS2 / A8 / 25/2013 implemented within the consortium BIOTRIGEN.

Poster 4.3

Identification of molecular markers linked to the leaf rust resistance gene (*Lr19*) in wheat (*Triticum aestivum* L.)

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Leaf rust disease caused by the fungus *Puccinia recondita* reduces quality and productivity of wheat throughout the world. Depending on the duration of infection and leaf rust resistance genes (*Lr* genes) the losses can reach up to 50%. The use of disease resistance genes is the most cost-effective and socially acceptable strategy to minimize the yield losses. The use of molecular markers has revealed as an effective and powerful approach complementing traditional plant breeding for improving crops. The application of molecular markers in plant breeding allows the controlled gene transfer and increase the efficiency of selection, which greatly reduces the time of release new varieties. This will enable to develop procedures for selection forms with the desired characteristics at an early stage of breeding. The aim of the study was to verify the suitability of the selected molecular markers for the identification of the leaf rust resistance gene *Lr19* in wheat genotypes. The plant material consisted of two genotypes carrying leaf rust resistance gene *Lr19* (GSTR 420, Agatha) obtained from National Small Grain Collection, United States Department of Agriculture, Agricultural Research Service Aberdeen - Idaho USA, five genotypes from Danko Hodowla Roslin Sp. z o.o. (T39, T68, T71, T74, TB), and three leaf rust susceptible genotypes (STHT 001, STHA 003, STHS 002). Analysis was performed using 4 primer pairs (*Gb, Xwmc221, SCS265, SCS253)*. The PCR reactions were set up with the recommended protocol for each primer pair. Of the four markers located on the chromosome 7DL, two were found to be linked to the gene *Lr19*. A microsatellite marker *Xwmc221* exhibited a codominant pattern, amplifying a 200-bp fragment from resistant genotypes GSTR420, Agatha, T39,T68, T70, T71 and a 220-bp fragment from susceptible genotypes. The STS marker *Gb* amplified a 130-bp fragment only from resistant genotypes confirming the results obtained from the analysis of primer pair *Xwmc221*. The other two pairs of primers (*SCS253* and *SCS265*) did not show desirable specificity. The obtained results showed that markers *Xwmc221* and *Gb* are suitable for testing wheat genotypes for the presence of leaf rust resistance gene *Lr19*.

The research is carried out in the project NCBR - PBS2 / A8 / 25/2013 implemented within the consortium BIOTRIGEN.

Poster 4.4

Association mapping in oats

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Oat is a hexaploid species with complex genome that limits the amount of available genetic maps. Lack of saturated consensus maps makes marker identification useful for breeding purposes difficult. This limitation could be partially overcome with association mapping approach.
In association mapping (AM), diverse mapping population is required, represented by individuals differing in the traits of interest. Plant materials undergo phenotyping and genotyping followed by identification of the extent of association of the markers with traits. The most associated markers are considered as putative markers of the trait(s) or QTLs.
The given study was based on 300 cultivars and lines of oat used in modern breeding programs. During vegetation period, plants were phenotyped. Earliness and plant height were assessed. When plants maturated, 10 panicles were taken from each field. The length of panicles, number of spikelets and seeds, seed weight and the percentages of husk in seeds as well as spikelet fertility and 1000 kernel weight (TKW) were determined. Earliness, height and TKW were used for association mapping studies. All analyzed plant materials were genotyped with DArT markers technology. Multiple Linear Model was applied for AM.
More than 80 DArT markers associated with the traits of interests were identified. Some of the markers were associated with more than one trait.
Using AM approach markers that could be used for marker assisted selection purposes were selected. These markers could also be combined on a single microarray plate and used for fast screening based on DArT technology eliminating phenotypic stage.

Poster 4.5

Identification of potential markers for dwarfing *Dw6* gene from oat

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The major purpose of cereal breeding programs is the development of modern cultivars characterized by high quality and increased yield of seeds as well as biotic and abiotic stress tolerance. Abundant yield could be achieved via cultivation of intensive forms that positively react towards complex plant protection and increased fertilization. However, in the case of lodging effective plant vegetation is diminished. Moreover, lodging leads to problems with harvesting, negatively influencing seed quality and affecting yield as well as decreases straw utility. In foreign as well as in Polish oat breeding programs dominant dwarfness *Dw6* gene is used to shorten a straw. Forms with the gene are usually about 60% shorter than identical materials without that gene, however, without loss of yield and seed quality.
The primary aim of the given study was the identification of putative markers towards *Dw6* gene by means of RAPD markers in combination with BSA approach. The F_2 biparential oat mapping population (STH 9787 x 'Bingo') was used. The STH 9787 is a line with *Dw6* gene that has short straw, while Bingo is the most prospective Polish breeding form of oat growing up to 100cm high. RAPD reactions were performed on bulks combined with the DNA of short and high F2 plants in parallel with the DNA of parental forms. About 300 RAPD primers were tested. Only three primers (K10, M05, N12) amplified products that were present in STH 9787 and the bulk of short F2s but not in the remaining cases. The identified fragments were considered as putative markers to the *Dw6* gene. The N12, M05 and K10 amplified putative markers that were 620, 840 and 1040 bp in length respectively. Only N12 was capable of amplifying the marker that was present among homozygous short lines with *Dw6* and was not identified among high plants.

Poster 4.6

Identification of effective oat crown rust resistance genes

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Crown rust is one of the most frequent diseases in oats caused by *Puccinia coronata* Cda. f.sp. *avenae* P. Syd. & Syd. Uredinia are linear, orange, and occur mostly on the leaf blades. Telia are mostly linear, black to dark brown, and are covered by the host epidermis. The pathogen occurs worldwide infecting both wild and cultivated oats, but its special form infects many grasses and barley.
By now, more than 100 *Puccinia coronata* resistance genes were described. Genes responsible for resistance were identified in *A. sativa* and its wild relatives (*A. sterilis, A. barbata, A. maroccana, A murphyi* and *A. strigosa)*. Complete resistance to specific *Pc* races is usually determined by single dominant genes. Less frequently, two or more genes may confer the resistance simultaneously.
Breeding cultivars with a crown rust resistance gene is the most effective method for controlling this fungal disease. Because of this, the most important is to use in oat breeding programs resistance genes that are effective in relation to the existing population of *Puccinia coronata* Cda. f.sp. *avenae*.
In Europe, the genes originating from *A. sterilis*, namely *Pc50*, *Pc59*, *Pc60* and *Pc61,* remain still effective. However, some races of *Puccinia coronata* overcame the resistance determined by *Pc39* and *Pc68* genes. The systematic studies related to the identification of effective *Pc* genes in the case of materials bred in Poland have not been performed for more than 20 years.
Basing on studies performed on differential lines with crown rust resistance genes it was demonstrated that lines *Pc52*, *Pc91* and *Pc104* were completely resistant to all *Puccinia coronata* races collected in Poland. These resistant lines could be used in oat breeding programs to improve the level of crown rust resistance.

Poster 4.7

Molecular characteristics of oilseed rape genetic resources collection from the IHAR-NRI, Research Division in Poznań

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The present work is concerned with determining DNA profiles of agronomically important oilseed rape cultivars from genetic resources collection of the IHAR-NRI, Research Division in Poznań.
Fifteen doubled haploid lines were chosen basing on their seed yield and quality traits, as seed oil and glucosinolate content, fatty acid profile in seed oil and also protein and fiber content. Genomic DNA was isolated and analyzed with a set of 35 microsatellite primer pairs; PCR amplification products were separated by capillary electrophoresis on an ABI Prism 3130XL Genetic Analyser (Applied Biosystems) and scored with the use of the PeakScanner software. Genetic relationships and degree of similarity among individuals of the collection were established using the GenStat statistical package, whereas 97 polymorphic markers were identified.
The obtained genetic similarity corresponded to the pedigree relationships among the plant material. Further analyses will be performed in order to determine DNA profiles as well as the structure of population comprising economically valuable winter oilseed rape cultivars, breeding lines and F1 hybrids. Association studies will be subsequently undertaken for development of molecular diagnostic test kit for MAS pedigree breeding.

Poster 4.8

Genetic variability in two DH line populations of oilseed rape (*Brassica napus* L.) obtained from reciprocal crosses between black- and yellow-seeded DH lines

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Today, oilseed rape is important source of high nutritional quality oil with a balanced fatty acid composition, a valuable material for many industrial branches and a protein-rich meal for livestock feed. The yellow-seeded oilseed rape has thinner seed coat and thus higher oil and protein content as well as lower fibre content than black-seeded types. Therefore, the development of *Brassica napus* with yellow seed colour is an important aim for oilseed industry.
Yellow-seedness does not exist naturally within species *B. napus*. Breeders try to develop yellow-seeded oilseed rape through transfer this trait from related species, such as *B. rapa, B. carinata*, *B. juncea* or via resynthesis of *B. napus* from yellow-seeded *B. rapa* and *B. oleracea*. However, the introduction of this trait to oilseed rape involves reduction in yield and lowering of agronomic performance. Therefore yellow-seeded breeding materials are improved by further crossing with very well yielding black-seeded forms.
The material for this study consisted of two doubled haploid populations of winter oilseed rape obtained from F_1 hybrids of reciprocal crosses between black-seeded DH line H_2-26 and yellow-seeded DH Z-114 line derived from natural mutant with bright seeds and spring line of *B. napus* with segregating seed colour, and the parental forms. The population, marked HZ, consisted of 19DH lines, derived from a F_1 hybrid, DHH_2-26× DHZ-114. The population marked ZH consisted of 25 DH lines, derived from a F_1 hybrid, DH Z-114 × DHH_2-26.
The aim of this study was to estimate the diversity of doubled haploids in terms of yield, yield structure and some quality traits, to determine a correlation between the studied traits and their heritability and make a clusters of the studied objects in terms of several features together as well as selection of the best yellow-seeded genotypes.

Poster 4.9

Dinitroaniline and phosphorothioamidate herbicides as alternative chromosome doubling agents for triticale haploids

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To be successful, the doubled haploid breeding approach requires an effective production system. Androgenesis is the most popular system of DH production in triticale; it appears to be efficient and reliable for most genotypes. However, since only ca. 20% of regenerants are spontaneously doubled haploids, for a majority of regenerants a successful and reliable method for doubling of the chromosome numbers is still an indispensable step. Traditionally, colchicine has been the most trusted compound for chromosomes doubling. Unfortunately, it is considered to be toxic: it inhibits cells proliferation, reduces the regeneration potential, induces chimaerism, hence its use should be avoided. From this reason, several other antimitotic compounds are currently being tested in a range of experiments and on several different species. Dinitroaniline and phosphorothioamidate inhibit microtubule assembly in a manner comparable to colchicine which makes them feasible alternatives. Their advantages over colchicine are related to lower toxicities, especially as relatively lower working concentrations are needed, and they exhibit high polyploidization capacities because of their highly specific binding with plant tubulins.
The purpose of this study was to determine the application regimes *in vitro,* including concentrations and application timing, as these affect the survival rate of regenerants and the efficiency of chromosome number doubling (flow cytometry measurements). Additionally, the effect of doubling agents on morphology of regenerants was examined. The experiments were done on standard breeding hybrids and model cultivars of winter triticale produced via androgenesis in routine anther culture procedures. Results show an increase in the proportion of DH plants compared to the spontaneous doubling rate, but no increase over the chromosome doubling rate by the standard in vivo application of colchicine

Poster 4.10

Proteins associated with efficient androgenesis initiation in low temperature-treated microspores of triticale (× *Triticosecale* Wittm.)

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Microspore embryogenesis (androgenesis) is an alternative developmental pathway characteristic for immature male plant gametophyte. The process, triggered by specific stress treatment results in formation of haploid (n)/doubled haploid (2n; DH) plants, highly advantageous in many research areas and breeding practise. However, deployment of DHs technology depends mainly on its effectiveness, which is for many species, among them – triticale, insufficient for practical purposes. Many studies have been focused on identification of parameters important for effective induction of androgenesis, but a lot of questions still remain. Four DH lines of winter triticale used in the study (two recalcitrant: DH19, DH72 and two responsive: DH28, DH47), were selected from the mapping population "Saka3006"×"Modus" according to their androgenic responsiveness evaluated by anther culture method. Since cold treatment (3 weeks at 4°C) occurred to be the most efficient in triticale androgenesis induction, the protein profiles were analysed in anthers collected from freshly cut (control) and cold-treated tillers. The proteins were isolated according to the phenol-based procedure (Hajduch et al. 2005). The protein expression patterns were examined by using 2-D electrophoresis. Spots up-regulated more than 2 fold were chosen for identification by MALDI TOF/TOF MS/MS analysis. Cold treatment induced changes in abundance of protein species. Protein spots which were up-regulated were more abundant as those which showed down-regulation. The identified proteins were mainly associated with metabolism (beta-amylase), stress response (heat shock 70 kDa protein) or cell energy management (ATP synthase subunit beta). What is more, the expression of some proteins was specific for high or low androgenic responsiveness. According to our knowledge, there is the first report concerning the identification of proteins involved in the regulation of androgenic responsiveness in triticale.

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Poster 4.11

Stress-induced changes required for effective initiation of microspore embryogenesis in triticale (× *Triticosecale* Wittm.) anther cultures

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Microspore embryogenesis (ME) is an alternative, sporophytic pathway of microspore development initiated as a response to various stress treatments. The resulting haploid (n) / doubled haploid (2n; DH) plants are highly valued in many research areas and in breeding practise. However, the precise mechanism of the process and the factors determining high effectiveness of DH production have not yet been identified. In order to gain a better understanding of the mechanisms controlling triticale ME, the generation of reactive oxygen species (ROS), antioxidative system activity, and disturbances in hormonal homeostasis were analyzed in ten DH lines of winter triticale as the most universal and dynamic changes in response to stress conditions. The DH lines used in the study were selected from the mapping population of 'Saka 3006'×'Modus' as significantly different in ME responsiveness. The analyses were conducted in the anthers collected from freshly cut tillers (FC) and then from cold-treated tillers (3 weeks at 4°C; CT), in which microspore reprogramming had been initiated. Low temperature treatment significantly increased H_2O_2 accumulation and changed the level of all analyzed phytohormones. Even excessive generation of H_2O_2 did not endanger cell viability as long as the cells exhibited high activity of H_2O_2-decomposing enzymes – catalase and peroxidase. The role of these antioxidative enzymes cannot be replaced by non-enzymatic antioxidants. An important prerequisite for effective ME was a specific hormonal homeostasis as anthers of highly embryogenic DH lines after ME-initiation treatment contained higher concentrations of IBA, *trans* zeatin and ABA but lower amount of IAA in comparison with recalcitrant genotypes. In conclusion, genetically controlled but environmentally modified cell tolerance to oxidative stress seems to play an important role in triticale ME, which is then regulated by complex and concerted PGRs crosstalk.

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Poster 4.12

Evaluation of the ability to androgenesis of rye (Secale cereale L.) F_1 hybrids

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Androgenesis by anther culture is one of common methods used to obtain doubled haploid lines (DH) of rye. The production of doubled haploids in the *Secale* genus is still laborious, has low efficiency and depends on many factors. The aim of the experiment was to assess the capacity to induce androgenesis in an anther culture established from F_1 hybrids and to assess the impact of the genotype and applied induction medium. Fifteen winter rye F_1 hybrids obtained from DANKO Plant Breeding were used for anther culture. The modified protocol for rye described by Immonen and Tenhola-Roininen (2003) was used in the experiment: induction media 190-2 (Xingzhi and Han, 1984) supplemented with 2 mg/l 2,4-D and C17 (Wand and Chen, 1983) in two variants: with the addition of 2 mg/l 2,4-D and with the addition of 1 mg/l 2,4-D and 1 mg/l of dicamba. The anther culture experiment consisted of 10 replicates (100 anthers from 1 spike cultured in the same Petri dish, diameter 90 mm) for each medium and genotype tested. 10 spikes from different F_1 rye hybrid plants were used for each of the three induction media. The best results were obtained on medium C17 with 1 mg/l 2,4-D and 1 mg/l of dicamba (the average efficiency of androgenesis induction for all 15 rye hybrids – 5.4%), whereas the lowest efficiency was noted with medium 190-2 supplemented with 2 mg/l 2,4-D (4.9%). Androgenesis was observed in all of the F_1 rye hybrids under study. The high capacity to induce androgenesis was observed in proper F_1 rye hybrids: 572/12 (33.2%), S1188/12 (21.0%), S1194/12 (14.0%). The highest number of green plants was regenerated on medium C17 with 1 mg/l 2,4-D and 1 mg/l of dicamba – 46 plants, from three F_1 rye hybrids (572/12 – 18 plants, S1194/12 – 12 plants and S1188/12 – 11 plants). In the experiment 160 plants were obtained from anther culture, including 75 green plants (46.8%) and 85 albino plants (53.2%). The ploidy level of the obtained green plants was different: 13 of the plants tested by flow cytometry were haploids, 46 plants were diploids and 4 were tetraploids.

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Poster 4.13

The effect of auxin transport inhibitors and exogenous auxins on microspore embryogenesis in *Brassica napus* L.

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Auxins, indole acetic acid (IAA) and indole butyric acid (IBA) are physiologically relevant natural types of auxin that have been implicated in plant embryogenesis. While, IAA is the most intensively studied, the role of IBA remains still to be elucidated. Recently, the role of endogenous IAA and IBA in pollen formation and androgenesis initiation was postulated. To verify this hypothesis, an approach to quantify endogenous IAA and IBA level in *in vitro* cultured isolated microspores (IM) was undertaken. Additionally, (1) the place of free IAA accumulation within microspore, and (2) the effect of auxin transport inhibitors and exogenous auxins on the embryogenesis induction effectiveness were investigated.
In the study, two rape-seed spring genotypes (cv 'Campino' and 'DH4079' line) differed in their capability to androgenesis were used. Endogenous IAA and IBA were analysed by common chromatographic technique in IM under various *in vitro* treatments (18°C, heat shock, auxin transport inhibitors or exogenous auxins). Auxin was visualised by 'whole mount' immunolabelling procedure.
Although, the endogenous IAA/IBA levels in microspores changed under applied *in vitro* treatments, it had no impact on embryogenesis effectiveness in recalcitrant cultivar. What is more, it even inhibited androgenesis for responsive DH line. In general, culture medium supplementation with exogenous IAA/IBA elevated endogenous IAA/IBA levels in IM being under both temperature treatments (18°C, 32°C). Auxin transport inhibitors reduced internal IAA and IBA levels, when IM were heat-treated.
Heat shock occurred to be prerequisite for effective microspore embryogenesis in *B. napus*. Its effect couldn't be replaced by exogenously applied auxins. Moreover, when endogenous IAA/IBA levels exceed the optimum at the moment of microspore isolation, embryogenesis is inhibited or even blocked.

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Poster 4.14

Regeneration of carrot protoplasts in presence of NaCl in culture medium

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Salinity in soil is one of the major abiotic stress reducing plant growth and productivity worldwide. The deleterious effect of salinity on plant growth is associated, among others, with toxic influence of ions – mainly Na^+ and Cl^-. In this regard, many strategies are being developed for new crop varieties development including *in vitro* selection of the existing crop germplasm.
The main objective of the present study was to evaluate salt tolerance in carrot protoplast cultures. Protoplasts of three accessions including open pollinated cultivar 'Dolanka' and two landraces from Iran (DLB-A and NLB-A) were isolated from yang leaves according to a previously described protocol (Grzebelus et al. 2012). Protoplasts immobilized in calcium alginate were exposed to different concentrations of NaCl (0, 10, 25, 50, 100, 200, 300, 400 mM). To assess the effect of NaCl on protoplast growth, the ability to form aggregates (so-called plating efficiency) was estimated after 10, 20, and 40 days of culture. After two months of continuous salt treatment from developed callus and proembryonic mass plants were regenerated according to Grzebelus et al. 2012.
The yield of released protoplasts was high and varied on average from $2.8 \pm 0.3 \times 10^6$ per g of fresh weight to $5.9 \pm 0.5 \times 10^6$ for 'Dolanka' and NLB-A, respectively. The Iranian landraces showed approximately two times higher isolation efficiency in comparison with 'Dolanka' ($P < 0.001$). Just after isolation protoplasts of all accessions exhibited similar cell viability reaching on average 70 %. Presence of NaCl in culture medium strongly influenced on growth of carrot protoplasts. Salt concentrations higher than 50 mM decreased plating efficiency in comparison with control treatment both in 10-, 20- and 40-day-old cultures ($P < 0.001$). After application of 200-400 mM of NaCl complete arrest of mitotic divisions was observed. Plants were regenerated from 0-50 mM or 0-100 mM NaCl-treated cultures for 'Dolanka' and both Iranian landraces, respectively.

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Poster 4.15

In vitro cultures of *Medicago truncatula*

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Medicago truncatula Gaertn. is annual plant originated from Mediterranean region and is commonly cultivated for forage production. The species has become also model for legume biology studies due to its relatively small genome, short life cycle, diploidy and autogamous fertilization. For these reasons it is an interesting research object in molecular genetic studies. Moreover, *Medicago truncatula* produces secondary metabolites, especially saponins and flavonoids. Plant biochemical profile can vary depending on growth conditions while *in vitro* cultures enable to maintain stable growth conditions.

The presented experiment was to work out a method of obtaining callus tissues which may be used for synthesis of secondary metabolites in *Medicago truncatula* tissues under *in vitro* conditions. The initial material were seeds of *Medicago truncatula* cv. Jemalong A17 which were sterilized with ethanol and hydrogen peroxide, then placed in Petri dishes on Linsmayer & Skoog (LS) medium for germination. Seedlings were transferred to Erlenmayer flasks on the same medium. The leaves of *in vitro* plants were the material for callus induction. Explants were put on two variants on LS medium: enriched with 0.5 mg/l 2,4 D (2,4-Dichlorophenoxyacetic acid) and 1 mg/l BAP (6-Benzylaminopurine) or with 1 mg/l 2,4 D and 1 mg/l kinetin. Simultaneously pH of the media used to obtaining callus tissue was 5,8. Petri dishes with explants were stored in a growth chamber applying photoperiod 16/8h and temperature 24°C.

Callus tissue formed on both media after 4 weeks and was friable, cream or light green. The induction of callus tissues on media containing 1 mg/l 2,4-D and 1 mg/l kinetin was more efficient than on the other one. Callus formation was observed on all explants and nearly half of calli were abundant. In contrary, in the case of application of 2,4-D and BAP many explants showed necrosis and formation of abundant callus was noticed only on few of them.

Obtained results allowed to select the proper media composition for callus induction and biomass production for inducing suspension culture.

Poster 4.16

Effectiveness of combining two transgenes in tobacco hybrids in protection from potato virus Y

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Genetic transformation enables to insert genes originated from phylogenetically remote organisms. Plants resistant to viral diseases can be obtained by transformation with genes coming from viruses. Pathogen–derived resistance is conditioned by a coat protein gene or viral RNA. Tobacco breeding lines containing lettuce mosaic virus (LMV) coat protein gene as well as lines with potato virus Y (PVY) replicase gene were obtained in Institute of Soil Science and Plant Cultivation. Transgenic lines showing high resistance to different PVY isolates were crossed in order to obtain hybrid lines containing both transgenes in one genome. In this study we present the effectiveness of transgenes combined by crossing line MN 944 LMV containing gene of LMV coat protein (LMV CP) with line AC Gayed ROKY1 containing gene of PVY replicase in sense orientation (ROKY1) and with line AC Gayed ROKY2 containing gene of PVY replicase in antisense orientation (ROKY2). Hybrid plants were analyzed by PCR to detect the presence of transgenes. Individuals containing two transgenes were inoculated with PVY under greenhouse conditions. The presence of two transgenes was confirmed in over 50 % of hybrid plants. All individuals in generation F_1were resistant to a mild PVY isolate and some of them were resistant to a stronger isolate. Generations F_2 and F_3 were obtained from a hybrid form coming from crossing lines AC Gayed ROKY2 and MN 944 LMV. The line showed high level of resistance to strong isolates of the virus.

Poster 4.17

TALEN-induced mutagenesis to knock-out transgenes in Arabidopsis

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Targeted genome editing, mediated by engineered nucleases, has emerged as a genetic tool to improve crop plants and ensure sustainable food production. Moreover, gene targeting based on TAL effectors provides gene modifications and up to now is not considered as GMO approach. Precise editing of DNA sequence is possible by introducing double strand break (DSB) through designed site-directed nucleases. Transcription activator-like effector nucleases (TALENS) have been shown as a breakthrough technology in plant research. TALENs are customizable fusion proteins between a specific DNA binding domain and FokI endonuclease that induce DSBs. This invoke cellular DNA repair mechanism that can be exploited to generate small insertions, corrections or deletion for gene knockouts, or integration of a template for targeted gene replacement. We describe the outline of our study to apply TALENS for reporter sGFP-KDEL and GUS transgenes silencing in *Arabidopsis thaliana*. The transgenic lines of Arabidopsis harboring pYF133 and pGH217 vectors were obtained by the floral-dip transformation method using *Agrobacterium tumefaciens* AGL1 strain. The assembly of specific TALENS for transgenic loci and the evaluation of potential off-target cleavage sites is described.

Poster 4.18

Influence of several biostimulators on embryological development and florescence of common buckwheat

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Productivity of common buckwheat is still a problem and is depended on such environmental factors as cold in the spring, water deficit during seedling and flowering stage as well as summer heat. Low fertilization rate has been widely recognized as one of the important causes of low productivity of common buckwheat. The main reason of poor seed setting is disturbance in development of embryo sacs and low viability of pollen. Buckwheat is characterized by strong selfincompatibility and is fertilized by bees and dipterae. Insect attracting depends on nectar abundance and its composition. Up to day many research undertaken in order to improve the productivity of seeds are unsuccessful. The aim of the work was to investigate if several biostimulators influence gametogenesis as well as number of flowers of common buckwheat. The experiment was performed on two Polish common buckwheat cultivars: 'Kora' and 'Panda'. Plants were cultivated in pots under open foil tunnel. In the study, following biostimulators were used: NAA, GA_3, BAP, NaCl, putrescine, cysteine as well as commercial products Tytanit and ASAHI SL. Stimulators were applied on plants at the beginning (I term) and during full flowering phase (II term). Control plants were treated with distilled water. Two weeks after stimulator treatment number of flowers was analysed. Moreover, flowers were collected for embryological analyses and analyses of nectar composition (using HPLC method). First results showed very high pollen viability of both studied cultivars: of both the control plants and treated with biostimulators. In most cases application of stimulators increased number of flowers per plant. Plants of cv. 'Kora' demonstrated more flowers under influence of putrescine and BAP applied in the beginning of flowering, while Tytanit and NaCl when used in the full flowering phase. 'Panda' plants showed higher number of flowers under influence of NaCl applied in the first term but cysteine in the second term. The term of stimulator application was also important for flower production. Both studied buckwheat cultivars demonstrated higher number of flowers after stimulator treatment in the full flowering stage.

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Poster 4.19

Immunolocalization of pectin and arabinogalactan protein epitopes in unpollinated ovules of *Beta vulgaris* L. genotypes

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The effective production of haploids and doubled haploids by the use of unpollinated ovules embryogenesis depends on many factors, from which the donor plant genotype seems to be the most important. But the mechanisms which determine the gynogenesis induction in particular sugar beet (*Beta vulgaris* L.*)* genotypes are still unknown. The presence and changes in plant cell wall composition have previously been described in relation to morphogenetic potential. Especially pectins and arabinogalactan proteins (AGPs) are the major cell wall components implicated to the development and differentiation of plant cells and tissues. Above mentioned compounds are widely distributed throughout the plant kingdom and occur either in intercellular spaces, plasma membranes and certain cytoplasmic vesicles. The aim of this study was to compare the occurrence and localization of pectin and arabinogalactan protein epitopes in unfertilized ovules isolated from *Beta vulgaris* L. genotypes of different embryogenetic potential. Explants were received from the unopened floral buds of eight donor plant genotypes. The localization of specific oligosaccharide antigens was visualized by histochemical and immunocytochemical methods. The obtained results allowed to verify if the variation in cell wall composition was correlated with different gynogenetic potential in analysed *Beta vulgaris* L. genotypes.

Poster 4.20

Capillary zone electrophoresis as a method of identifying low molecular weight glutenin subunits

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For the rheological properties and baking quality it is very important a number and composition of gluten proteins: gliadins and glutenins. Glutenin proteins due to the molecular weight can be divided into high molecular glutenin (HMW-GS) and low molecular weight glutenin (LMW-GS). Protein synthesis of the second group (LMW-GS) is controlled by three genes located on one of short arms of chromosomes, loci *Glu-3*. LMW-GS generate 30% of the technological properties of wheat and constitute one third of grain storage proteins and approx. 60% glutenin. The main objective of this study was to identify low molecular weight glutenin subunits by capillary zone electrophoresis.
The study acquired 50 wheat varieties with determined composition of high molecular weight glutenin (HMW-GS) but of unknown composition of LMW-GS. In order to determine the composition of low molecular weight glutenin subunits were used on the apparatus to capillary zone electrophoresis (CZE). Electrophoresis were performed on MDQ Beckman-Coulter (USA) apparatus. Separations were performed on a capillary having an inner diameter 50 μm and length of 30.2 cm, at temperature 38^{o}C and a voltage of 11.0 kV. The optimum buffer was 0.1 M iminodiacetic acid (pH 2.75) containing 30% (v/v) acetonitrile and 0.05% (w/v) hydroksypropylmetylcellulose. Time of a single analysis was 18 minutes. Electrophoretic separation was also performed using an apparatus for SDS-PAGE. In order to identify genes of *Glu-A3*, *Glu-B3* and *Glu-D3*, which are responsible for encoding low molecular weight subunits of glutenins, PCR using allelospecific molecular markers were carried out.

Poster 4.21

Comparative metabolomics of medicinal plant *Kalanchoe* species

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Kalanchoe is a large genus of the family Crassulaceae including more than 140 species, belonging to two sections *Kalanchoe* and *Bryophyllum.* Many of *Kalanchoe* species are widely used as indoor and outdoor ornamental plants. Moreover, the species possess an array of medicinal effects. They are used in folk medicine as stomach pain relief, against gastritis, diarrhoea, bilharzias, dysmenorrhoea, liver disorders, fever, female infertility, snake and scorpio bites, and general tiredness (Quazi Majaz et al. 2011). Furthermore, pharmacological activities (such as antidiabetic, antioxidant, immunomodulatory, and antiallergic, antiviral, antitumoral, hepatoprotective and antithrombotic) of several *Kalanchoe* species have been reported (Quazi Majaz et al., 2011). However, little is known about bioactive components in this plant material, making the standardization of this traditional drug difficult. Thus, comprehensive studies may contribute to evaluation of leaf extracts from the species. Our mass spectrometry analysis using HPLC-ESI-MS^n system in 18 *Kalanchoe* species revealed richness and differentiation of health-benefit phytochemicals. Alkaloids, triterpenes, glycosides, flavonoids and bufadienolides were identified in particular species.

In addition, chemotaxonomic analysis of all 18 studied genotypes was conducted on the basis of secondary metabolites UV profiles obtained by UPLC-PDA system. The chromatograms were mathematically pre-processed to form suitable to statistical analysis. Generated dendrograms revealed phytochemical relationship and degree of similarity in health-benefit compounds among all studied species. Such analyses are often used as markers for the quality control enabling the identification of many species.

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Drought tolerance - POLAPGEN-BD project open workshop

Oral presentation

Present and future agrometeorological conditions of the crop of spring barley in Poland

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Changes in the structure of vegetation in Poland together with global climate changes observed in recent decades have led to the deterioration of water conditions in rural areas in Poland. This raises the threat to agricultural crops, especially for spring cereals. Characterizing the spatial and temporal moisture conditions in agricultural areas in the whole country is the natural background for these researches. Particularly unfavourable phenomenon is decreasing the ratio of summer precipitation to winter precipitation, and increasing incidence of drought. Especially important is to determine the water shortages in the period from April to July. The probability of water shortage (the difference between precipitation and real evapotranspiration of barley crop) for the whole country during the reference period (1961-1991) and during the projected period (2061-2091) will be presented in the lecture. Calculations were performed for a regular grid of squares (515 cells covering the entire country), for each decade in the period from April to July - the growing season of spring barley. The values of meteorological elements for both periods generated by the model MPI-M-REMO which has the best compatibility with the actual data for the area of Polish were used as input. Average ten-day values of heat balance components for spring cereals have been calculated on the basis of meteorological data and the development phase of the plants for each cell and for both analyzed periods: the reference and projected. Average values of ten-day precipitation (P) and the latent heat of evaporation (LE - in the heat balance) were used to calculate of water shortages (P-ETR). Analysis of variability of water shortages is presented with respect to entire country and to selected 5 regions in Poland in order to capture the regional variation. Statistical analysis concerned the variability of precipitation, evapotranspiration, and real water shortages. During the vegetation period of spring barley in both periods (reference and projection) the precipitation are similar, except that in the projection period precipitation will increase in April and early May, but will decrease in period from 2 ten-day of June to the end of July. Pairing in the projection period will increase, which will result in an increase in water shortages. In the country scale, the worst conditions prevail and will be prevailed in central and eastern Poland and in Kujawy, while the best in the north-eastern Poland. From the point of view of the water conditions, June and the first decade of July is a critical time for the cultivation of barley in both periods, but the risks will be greater in the projection period. There is only a 10% probability that the shortage of water in the critical period in the most vulnerable areas does not exceed 10 mm, and 50% probability that it does not exceed 50 mm.

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Oral presentation

Tolerance of spring barley lines to temporal drought stress in relation to grain yield and yield components

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The subject of the study was to determine the response of several spring barley lines derived from parental forms originating from different climatic zones to temporal drought stresses. Grain yield and yield components: productive tillering (number of fertile tillers), number of grains per spike and weight of 1000 grains (WTG) were considered as the indicators of their response.

Experiments have been carried on in 2011-2013 years at the greenhouse of Grabow ES (E 21° 39', N 51° 21') of the Institute of Soil Science and Plant Cultivation – State Research Institute. The pots were filled with the mixture of loamy soil (7 kg) and sand (2 kg) characterized by 23% w/w WHC, sufficiently supplied with all nutrients. The population of two hundred forty lines derived from parental forms Maresi (Germany), Harmal (Syria) and Georgie (Great Britain) and 60 varietes registered in Poland was tested against short-term drought stresses introduced at the tillering stage (BBCH 23, 31 days after sowings) for 11 days or at full flag leaf stage (BBCH 45-47, 50 days after sowings) for 14 days. In the control treatment, soil moisture was maintained at the optimal level of 65% WHC for the whole vegetation period, and in the treatments S1 and S2, at the level of 35% WHC.

The data concerning barley yield from the pot and all yield components have been processed by means of cluster analysis, separately for the drought stress applied at tillering stage and at flag leaf stage. On the base of the analysis, two clusters of barley genotypes have been distinguished. Against the first stress, 31% of barley genotypes proved to be tolerant and 69% sensitive, while at the second stress, the share of tolerant genotypes was 14% and the sensitive ones 86% respectively.

The differences between both groups of genotypes can be partly explained by the compensation processes among the yield components. Genotypes tolerant to the stress at tillering stage responded to this stress by increasing number of productive tillers, while the number of grains per spike and WTG stayed practically unchanged. Sensitive genotypes reduced number of grains per spike and WTG, which were not compensated by the number of productive tillers. The mechanism of tolerance against the stress at flag leaf stage was somewhat different. Tolerant genotypes compensated successfully the decrease of number of grains per spike by significant increase of WTG, while all yield components of sensitive genotypes decreased substantially under this stress.

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Oral presentation

Mapping of QTLs for the plant height and yield forming traits in RIL population of spring barley (*Hordeum vulgare* L.) under various environments

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Drought is one of the major environmental stresses constraining barley growth and productivity. A population of 100 recombinant inbred lines generated from the cross between Maresi and Cam/B1/CI08887/CI05761 (originated from Europe and Syria, respectively) were grown in the greenhouse experiments in optimal and water shortage conditions. At the maturity seventeen agronomic traits were measured, and the heading date was recorded during the vegetation. The genetic maps consisting of SSR and SNP markers were constructed. Lines were significantly differentiated with respect to studied traits. A total of 153 quantitative trait loci (QTL) affecting analysed traits were identified. The plant height, considered as the length of main stem, as one of the most important agronomic traits and strongly affected by drought, will be particularly discussed in this presentation. Co-segregation of the QTLs for plant height and grain yield (understood as the grain weight per plant) was investigated. Eight QTLs controlling the plant height were detected. In half of them Maresi contributed the allele reducing the trait. The strongest QTL ($-Log_{10}$(P-value) = 12.5) was identified in the 3H.1 linkage group (105.75 cM) at the position where the *denso* locus was previously mapped. Traits and QTLs which were identified around the *denso* locus were analysed. Stability analysis was performed for the grain weight per plant to select genotypes with short plant stature (inherited from one of the parent) and simultaneously with acceptable grain yield under optimal as well as stressed conditions.

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Oral presentation

Contact angle and surface free energy of plant leaves and their changes under drought conditions

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Surface of plant leaves is hydrophobic due to the presence of cuticle, which is a mixture of waxes and cutin. The waxes consist of a wide range of fatty acids (carbon number from C_{16} to C_{18}), aliphatic alcohols, aldehydes, ketones (carbon number from C_{22} to C_{32}) and sometimes also triterpenes and sterols. They play two basic functions – protection of plants from an uncontrolled loss of water and reduction of the leaching of inorganic and organic compounds from leaves interiors. In the stress conditions the composition of cuticle could be rearranged which results in a change of the leaves surface wettability. Increase in the amount of wax on the leaf surface causes its hydrophobization, resulting in a plant resistance against drought.

Hydrophilic/hydrophobic properties of a surface are connected with its affinity to water. They can be characterized by the wettability, which is the result of interaction between solid and liquid phases. The surface free energy SFE (γ) depends on the type of forces on the surface of a condensed phase (solid or liquid): always existing dispersive forces (London's type) and the forces of polar nature (electrostatic between ions, permanent dipoles, induced dipoles, hydrogen bonds, bridges and acceptor-donor interactions). SFE is a very sensitive parameter to changes in the surface structure and chemical composition. Even very small changes in the nature of the surface can by reflected by SFE.

Typically, the surface of most plant leaves is hydrophobic due to the epicuticular waxes. Water contact angle for barley cultivars grown under control condition varied from 133.1 to 137.0˚, and under drought from 140.0 to 145.3˚. The increase in the value of the contact angle during stress shows plant defense to drought stress. Surface free energy of leaf decreases hence the surface becomes more hydrophobic.

The observed changes in the value of θ and γ_S are associated with physiological reactions in plants, which is the effect of change in the composition of the wax surface and reorganization of the structure of the surface. Obtained results are promising, SFE can be a rapid tool for assessment of leaf hydrophobization process under drought condition.

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Oral presentation

Hordeum vulgare calcium-dependent protein kinase 34 regulates drought stress response

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Calcium-dependent protein kinases (CDPKs) are structurally conserved serine/threonine kinases involved in multiple processes, including drought stress response. Our analysis revealed that *Hordeum vulgare* calcium-dependent protein kinase 34 (HvCPK34) is activated upon drought stress treatment. Therefore to understand the structural mechanisms of *its activation* at first we analyzed is cellular localization. To determine HvCPK34 protein localization *in vivo,* C-terminal GFP fusions were generated and transiently expressed under the control of the *35S* promoter in *A. thaliana* and barley protoplasts. Active and catalytically inactive (K94M) forms of HvCPK34 were predominantly localized in the plasma membrane. Interestingly, permanently active form of HvCPK34 was localized in the nucleus. To test the mechanism of HvCPK34 activation, recombinant HvCDPKs were purified and CDPK kinase activity was analyzed using *in vitro* and in gel assay methods. Overall, we demonstrate evidence for the new role of HvCPK34 in the regulation of drought stress response.

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Oral presentation

Drought-induced free proline synthesis and ABA accumulation in leaves and roots of spring barley genotypes of different origin

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The purpose of the study was to examine the effect of drought on proline and ABA accumulation, activity of Δ^1-pyrroline-5-carboxylate synthetase (P5CS), and expression of the *P5CS* in leaves and roots of two barley (*Hordeum vulgare* L.) genotypes: the Syrian breeding line Cam/B1/CI and the German cultivar 'Maresi'. The experiments were conducted in a climatic chamber (65-75% RH; 20/13°C day/night temperatures; 14/10h light/dark, 180 µmol m^{-2} s^{-1} PPFD). Soil moisture was kept at the level of field capacity (pF=2.2). Drought was imposed at 3-leaf stage by reducing irrigation. Soil moisture in pots of stressed plant was kept at the level of 8-6% (w/w, pF=3.2). The control plants were irrigated regularly. The middle part of fully developed leaf as well as root samples were collected from control and water deficit treated plants after 3, 6 and 10 days of stress for measurements water content, proline (1), P5CS activity and expression (2) and ABA content (3). Drought caused a gradual decline in leaf and root water content but greater decrease was observed in leaves than in roots. Free proline content in roots of both genotypes increased 6-10 fold within the first three days of stress and this raised level remained in the following days. Proline content in leaves increased only 2-4 fold during the first stress days and was much lower than in roots. After 10 days of stress the proline level in leaves was similar as in roots. Proline accumulation in leaves and roots of barley seedlings was preceded by the increase of *P5CS* gene expression. P5CS activity increased in both roots and leaves with the duration of drought stress. The increase of P5CS activity observed after 10 days of stress was preceded by the increase of *P5CS* gene expression. Drought caused gradual increase of ABA content in both organs. However, earlier increase was observed in roots of both genotypes. The obtained results show out that the activity of P5CS is controlled at the transcriptional level both in roots and leaves. However the examined barley genotypes did not differ significantly in proline and ABA accumulation under drought.

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Changes in metabolite profiles of barley (*Hordeum vulgare* L.) subjected to drought stress

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Drought negatively affects plant growth, development and survival, therefore it is one of the most important abiotic factors limiting crop yield. The aim of conducted research was the analysis of the metabolome changes in spring type barley subjected to controlled water-deficit conditions.
Metabolite profiling experiments of barley leaves and roots were carried out on four varieties: Maresi, Cam/B1/CI, Sebastian, Stratus and 100 recombinant inbred lines developed through single seed descent from a cross between Maresi × Cam/B1/CI. Analyses of barley metabolite extract samples were performed using 6890N gas chromatograph (Agilent) – GCT Premier mass spectrometer (Waters).
In the analyzed extracts of barley tissue 114 derivatives were detected, representing various classes of metabolites: amino acids, amines, organic acids, carbohydrates, polyhydric alcohols, fatty acids and sterols. By using TargetSearch – a package for the analysis of GC-MS metabolite profiling data – quantification of detected in barley leaves and root extracts derivatives (101 and 100, respectively) was performed. Plants exposed to water deficit revealed many changes in metabolite profiles in comparison with control plants, particularly in abundances of proline and 1-kestose.
Additionaly qualitative and quantitative composition of cuticular waxes of two barley genotypes: Maresi and Cam/B1/CI at optimal soil moisture and water deficit conditions was analyzed. In total 35 compounds present on the surface of the barley leaves were detected. The analyzes showed that the dominant components of the wax in this species are homologous series of long-chain aliphatic compounds: alkanols, alkanes and fatty acids. However, water shortage did not significantly affect the composition of the barley cuticular wax.

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A high density 'function map' aimed at the dissection of drought tolerance related QTL in barley

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The aim of the study was to construct a high density molecular map of barley enriched with various types of candidate genes (CGs), potentially involved in drought stress response/tolerance in barley. This, so called, a 'function map' is a useful genetic tool for the precise localization of quantitative trait loci (QTL) for drought tolerance-related traits and for the identification of linkages between these QTLs and CGs. It allows designating the most probable genes that are involved in determination of the increased tolerance to water deficiency in barley.
In order to create a 'function map' of barley, a consensus genetic map of three recombinant inbred line (RIL) populations derived from the crosses between European cultivars and drought-tolerant Syrian lines was constructed using simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) markers. A set of candidate genes with putative involvement in drought stress response/tolerance to be positioned on the map was selected from two different resources. Firstly, literature data and public databases were searched for functional and regulatory genes with known nucleotide sequences and reported associations with enhanced drought tolerance in model plants, such as rice or Arabidopsis. For the selected genes, the identification of genomic sequences of putative barley orthologs was performed based on sequence homology with known proteins across species. A second group of CGs consisted of novel genes with unknown molecular function, either up- or down-regulated in response to the drought stress, chosen based on the transcriptome profiling of the parental genotypes of RILs in a microarray experiment. For all the CGs, the identification of the polymorphism between parental genotypes of RILs was conducted, the genotyping procedures were developed, and the genotyping analysis was performed in RIL populations. The constructed, high-density consensus map, enriched with various types of candidate genes is designated for precise mapping of QTL for drought tolerance-related traits and for dissection of the most probable candidates genes that control them.

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Oral presentation

Identification of QTL associated with yield and earliness in barley RIL lines derived from hybrids between European and Syrian varieties differentiated in tolerance to water deficiency

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Water deficit is the most devastating abiotic stress on a global scale and barley is considered as an excellent model plant for investigating the genetic basis of drought tolerance in crops. In the present study highly diverse parents segregating for the trait of interest (earliness) – Lubuski (Europe) and Cam/B1/CI08887/CI05761 (Syria) were crossed and 100 F8 RILs were subjected to drought stress. The experimental approach using both genotype and phenotype data allowed the creation of saturated genetic map with SNP and SSR markers and localization of QTLs associated with yield-forming traits. The research aimed at the evaluation of different traits in plants grown both under controlled and stressed conditions. Cam/B1/CI08887/CI05761 heading time under controlled conditions was about 15-19 days shorter than for Lubuski and highly significant differences among lines were also observed. The influence of irrigation condition on the vegetation duration was noticed - drought caused delay of the development stages. QTLs in RIL population were found for many traits associated with spike morphology, plant architecture, grain and straw yield and development stages. It was found that number of tillers per plant increased under drought conditions and it seems to be an important method of adapting to changing environmental conditions. A total of 72 QTLs were detected on all barley chromosomes. Four QTLs for heading stage were mapped on chromosome 2H, 3H.1, 5H.3, 7H.2, respectively. The main QTL for HS was located on chromosome 2H, where the major photoperiod response locus (*Ppd-H1*) was reported. The SNP marker 5880-2547 (11_21015) was mapped close to the BOPA 2 SNP markers 12_30871 and 12_30872 which are located in *Ppd-H1*. Among all detected QTLs for particular traits, QTLs located close to this SNP marker were the most significant in the studied population. A set of 13 yield-forming traits were associated with QTLs detected in the earliness regions.

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Oral presentation

The functional analysis of candidate genes related to drought response in barley using TILLING strategy

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The TILLING strategy has been successfully used in functional analysis of genes related to various signaling pathways in many plant species. We have undertaken this approach in order to analyze selected barley regulatory genes related to response to drought: *HvABI5* (*Abscisic Acid Insensitive 5*), *HvDREB1* (*Dehydration-Responsive Element 1*), *HvDRF1* (*Dehydratation-Responsive Factor 1*), *HvSNAC1* (*Stress-Responsive NAC1*), *HvWRKY38*, *HvCBP20* (*Cap Binding Protein 20*), *HvCBP80* (*Cap Binding Protein 80*), *HvERA1* (*Enhanced Response to ABA 1*).
The main goals of our part of the project were as follows: (1) the identification of new alleles of candidate genes related to drought response in barley using TILLING strategy in *Hor*TILLUS population developed in our Department for spring barley cv. 'Sebastian', (2) the phenotypic and molecular analysis of mutants obtained from TILLING analysis, (3) the analysis of the mutation impact on the expression of the downstream genes in drought signaling pathway using the Agilent microarrays.
More than 130 mutations identified in eight drought related genes gave the average mutation density 1/322 kbp. More than 30 homozygous mutants were screened in drought assay in order to evaluate their response to water stress. Among them, six mutants characterized as tolerant to drought when compared to their parent variety wild-type – 'Sebastian' (*hvabi5.d*, *hvabi5.e*, *hvcbp20.ab*, *hvdrf1.a*, *hvdrf1.c*, *hvera1.b*) were selected for further analysis. Their response to drought was defined on the basis of RWC, chlorophyll *a* fluorescence and proline content measured at seedling stage in four time-points: (1) control growth, (2) adaptation to drought, (3) after 10 days of drought stress and (4) after 14 days of re-watering. Homozygous mutants were backcrossed with 'Sebastian' cultivar in order to clear their genetic background from other mutations. In order to analyze the impact of mutation (*hvabi5.d*, *hvabi5.e*, *hvcbp20.ab*, *hvdrf1.a*, *hvdrf1.c*, *hvera1.b*) on the expression of downstream genes the global profile of expression was analyzed using the Agilent microarrays. In the case of each mutant, genes that changed most dramatically their level of expression in response to drought stress were selected for further analysis.

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Oral presentation

The role of micro RNA in regulation of mechanisms leading to drought adaptation

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A number of miRNAs has been demonstrated to function in biotic and abiotic stress responses in plants. In this study, we analyzed the involvement of these regulatory molecules in response to drought stress in barley. We used Illumina deep sequencing technology to analyze the global miRNA expression level in severe drought (20% Soil Water Content – SWC) treated barley plants. We identified 304 conserved miRNAs in both, control and drought stressed plants. The expression levels of 120 miRNAs were down-regulated under drought conditions, 111 miRNAs were up-regulated, and the level of 74 remained unchanged.
We developed the RT-qPCR based platform to analyze the expression level of 140 pri-miRNAs in barley. This platform was used to monitor changes in expression level of barley primary miRNAs in minor (30% SWC) and severe (20% SWC) drought treated plants, as well as after rehydratation. We set the studied barley pri-miRNAs regarding their expression level during drought stress into three categories: a) unchanged, b) upregulated, and c) downregulated.
Additionally, we determined the structure of sixteen barley *MIR* genes (1,2). Barley miRNAs are encoded by genes with diverse organizations, representing mostly independent transcription units with or without introns. The intron-containing miRNA transcripts undergo complex splicing events to generate various spliced isoforms.
Drought is very often accompanied by heat stress, and these two combined stressful conditions are the major limitations to food production. Thus we were also interested in studying the heat–responsive barley miRNAs. Four barley miRNAs (miR160a, 166a, 167h, and 5175a) were found which are heat stress up-regulated at the level of both mature miRNAs and precursor pri-miRNAs (2). Moreover, the splicing efficiency of introns hosting miR160a and miR5175a is also dramatically increased in heat. Using the 5'RACE approach and degradome sequencing we identified and verified novel (*HOX9, NEK-5, ACC*) as well as conserved (*ARFs, PHV, REV*) target genes of barley miRNAs. Our results demonstrate transcriptional and post-transcriptional regulation of heat-responsive miRNAs in barley.

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Oral presentation

Analysis of calcium dependent protein kinase (CDPK) genes expression during drought adaptation in barley (*Hordeum vulgare* L.).

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The objective of this study was the analysis of expression of barley calcium dependent protein kinase (CDPK) genes in water deficiency conditions. Caryopses of two barley genotypes (tolerant Sebastian and susceptible Georgie) were subjected to osmopriming with 50mM $CaCl_2$, previously chosen based on a pilot experiment. Drought stress was imposed on three-week-old plants by withholding water and their leaves were taken at three selected time points related to field water capacity (pF= 3.2, 3.6 and 4.2). GeneChip Barley Genome Array 22K (Affymetrix) were used to perform gene expression profiling followed by qPCR analysis. The majority of barley CDPK studied genes showed distinct changes in patterns of expression during exposure to stress.
Additionally, genome-wide analysis of the effect of $CaCl_2$ treatment on drought adaptation in barley was performed. Analysis of variance set up groups of differentially expressed genes (DEGs) according to the studied factors and their interactions. Functional analysis of DEGs and their GO terms categorization was performed using Blast2Go. Enrichment analysis was done using χ^2 test at FWER (family-wise error rate) < 0.05 with Bonferroni approximation. MapMan and PageMan were used to visualize the cumulative results of our study. $CaCl_2$ treatment influence barley drought adaptation mainly through modification of gene expression of genes coding for PS light reaction and Calvin's cycle enzymes along with lectin protein kinase (LecRLK).

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Oral presentation

Differential analysis of barley leaves and root transcriptomes under drought stress and its application for molecular markers development

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Drought is one of the most limiting environmental factor for yield production in crop plants. It is therefore important to understand the mechanisms that allow the plant survival under decreased water availability. Changes in gene expression stand under the centre of plant response to drought and studies of transcriptomes allow to decipher the molecular basis of this response. The presented study was based on the differential gene expression analysis of two barley cultivars: Maresi and Cam/BI/CI, characterized by distinct response to drought. Seedlings on the developmental stage of three leaves were subjected to drought stress lasting 10 days and at that time the second leaf and root system were used to transcriptome analysis using Barley Gene Expression Microarray, Agilent. From 759 to 2904 genes were differentially expressed after drought, depending on the cultivar and tissue analyzed. Among them were genes involved in osmoprotectants synthesis, genes encoding proteins of abscisic acid response pathways, transcription, translation, RNA posttranscriptional processing, protein phosphorylation or vesicle and transmembrane transport. Among genes with the most prominent changes in expression level the genes for dehydrins and other proteins from LEA family, peroxidases, metallothioneins and heat shock proteins were found.
From the group of transcripts with the highest changes of gene expression after drought treatment a pool of 50 genes were subjected to the analysis of their genomic sequence polymorphism among three European barley cultivars: Maresi, Georgie, Lubuski and two Syrian cultivars: Cam/B1/CI and Harmal. The polymorphic sites were found in the fragments of 31 genes. Altogether, 253 polymorphisms were found in introns, exons and promoter sequences. The majority of changes were nucleotide substitutions, but some in/del sites with single or more nucleotides insertions/deletions were also noted. Additionally, one microsatellite site of (TA) motif was found to be polymorphic between 'Harmal' and other cultivars. The data on sequence polymorphism in genes with confirmed differential expression under drought may be used as a markers for their molecular mapping and for the study of their possible co-localization with QTLs related to drought response in barley.

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Oral presentation

Proteomic analysis of barley mapping population subjected to drought

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Plants as a sessile organisms are continuously affected by many biotic and abiotic factors. To survive harsh conditions they had to develop complex mechanisms. Drought is one of the severe abiotic factor that influence plants growth, development and productivity. Proteomics has become a very powerful tool in analyzing plant reactions to various environmental stimuli. Especially comparative studies are highly informative and provide insights into plant responses to a pre-determined stress factors, and in combination with other techniques it may increase the potential to discover biomarkers of enhanced stress tolerance. In present study a mapping population of spring barley (100 genotypes), which is a crossbred of European semi-dwarf cultivar Maresi and Syrian breeding line Cam/B1//CI08887/CI05761, was used to evaluate the response to drought on the proteome level. Two dimensional gel electrophoresis was used to monitor changes in accumulation profiles of the proteins. Identification of proteins affected by drought was carried out by mass spectrometry, MALDI-TOF and MALDI-TOF/TOF. Many of the drought-responsive proteins identified in the analysis were associated with photosynthesis, carbon and nitrogen metabolism, processes typically severely affected during water deficit conditions. Proteins involved in osmolytes biosynthesis, ROS scavenging, molecular chaperones and heat shock proteins also had changed accumulation profile. Most of the analyzed proteins appear to be a general stress indicator that differentiate only controls and plants subjected to drought. However, some of them discriminate also between tested genotypes and may be subjected for further analysis to reveal if there is a connection between their accumulation profile and degree of drought tolerance in plants, thus they may serve as a potential biomarkers.

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Oral presentation

Characteristics of the diversity of parental and reference lines of barley in terms of changes of the content of proline, sugars and ethylene under the drought stress

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Osmotic adjustment of plants involves the accumulation of various osmotically active molecules, including proline and soluble sugars, which lowers the osmotic potential of the cells in drought stress conditions and helps to maintain the cell turgor. Physiological function of proline has been recognized as to stabilize macromolecules, storage of carbon and nitrogen for use after relief of water deficit and regulation cellular redox status. An essential role of sugars in stress tolerance consists of not only direct involvement in the synthesis of other compounds and production of energy but also of stabilization of membranes and action as signal molecules. In the response of cereals to drought also substances of hormone character like ethylene are involved. Synthesized ethylene may serve as regulator and optimizer of the plant growth under stress conditions. The aim of presented experiments was to identify similarities/differences in the proline, sugars (mono- and di-carbohydrates) and ethylene content for two varieties of barley: drought-tolerant Cam/B1/CI and drought-susceptible Maresi (parental lines) and 99 reference lines (all received from the Institute of Plant Genetics in Poznań) during controlled drought stress treatment. Plants were cultured in an air-conditioned greenhouse at 20/17°C (day/night), and the humidity of the soil was stabilized to 3.2 pF. Soil drought (4.0 pF) was applied after the appearance of the 4th leaf or after the appearance of the flag-leaf and was maintained for 10 days. Contents of investigated substances were detected in the 2nd leaves (seedlings) and flag leaves of stressed plants and compared with results of the analysis made for control plants. Proline accumulation in control plants was at the same level for the Cam/B1/CI and Maresi in leaves of seedlings but in flag leaves, content of this substance was higher in Cam/B1/CI than in Maresi. For the other tested lines (for about 60% of them), the level of this substance was similar to this recorded for Maresi. Drought caused an increase in proline level, by about two orders of magnitude, in all genotypes, and the amounts of accumulated proline occurred to correlate with the ethylene synthesis. Among the carbohydrates, contents of mono-carbohydrates, glucose and fructose, were higher than 50% of the total analyzed sugars in all studied objects. In the investigated lines various amounts of individual sugars in both developmental stages were detected. The levels of glucose, registered in the investigated lines, were taken as a factor used for grouping plants and indication of similarity/differences in the accumulation of sugars in the stress conditions. Moreover, for parental varieties localization of radicals on carbohydrate molecules was detected (electron paramagnetic spectroscopy). It was concluded that content of sugars and sugar-radicals may be used to characterize stress tolerance of cereals.

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Oral presentation

Phenolic metabolites expression in leaves of barley inbred lines - comparison of greenhouse and field experiment

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One hundred inbred lines (RILs) of barley (*Hordeum vulgare* L.) grown in two experiments: in semi-controlled greenhouse and totally uncontrolled field conditions were subjected to metabolomic study. Significant differences in content of secondary metabolites between these two conditions were observed using two complementary mass spectrometers (high resolution Q-Exactive Orbitrap and an ion trap $MS^{n)}$. Relatively numerous set of metabolites (109 compounds) was identified in field grown plants - whereas 87 metabolites were found in the greenhouse experiment. Pattern of flavones glycosylation and acylation showed the greatest differences. Only 37 metabolites were common in both conditions. Among them hordatines and their glucosides, as well as blumenols derivatives deserved special attention.

In addition, advanced mathematical and statistical tools were used for pre-processing of the UV chromatograms of samples from both experiments in order to analyze differences between seedling and flag-leaf plants and between experiments with respect to metabolite level, as well as the interaction of RILs with time-points and conditions. In both experiments more than a half of metabolites changed their level during plant development. Interestingly, opposite expression of metabolites in development was noted in both experiments. In field most of compounds were up regulated (from seedling to flag-leaf stage) whereas in greenhouse down regulated.

Presented data showed great diversification and plasticity of barley metabolome depending on the growth conditions and timepoint of development. Utility of mathematical and statistical tools in comparative metabolomics allows precise study of all changes in the metabolites level in different conditions and in combination with a thorough phytochemical analysis may lead to further increase of knowledge on physiological processes in plant development.

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Oral presentation

Physiological response to water stress at seedling stage of spring barley lines

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Drought is considered the most important environmental factor that limits crop productivity, including barley. This problem occurs not only in the arid and semi-arid areas, but also applies to central Europe, where the rainfall varies from year to year. In Poland, the yield of spring barley can be strongly reduced both by spring (April–May) and summer droughts. Selection for grain yield under a representative low-yielding environment is suggested to be the most efficient strategy for improving yield stability of barley in arid or semi-arid environment. A number of physiological traits are proposed as selection criteria for drought tolerance.
We have examined the effect of drought stress on the physiological processes of seedlings of 99 barley lines obtained by the single seed descent (SSD) method. Soil drought (5.7%) was introduced at the 4th leaf stage (16 d after emergence) and was maintained for 10 days. The research concerned (a) water relations of seedlings and integrity of cell membranes, (b) a fast chlorophyll *a* fluorescence kinetics, (c) gas exchange, (d) photochemical activity of PSII, (e) analysis of antioxidant system built with tocopherols, beta-carotene and selected antioxidant enzymes. SSD lines of barley showed strong variation of tolerance to drought stress at the seedling stage. These changes mirrored in all analyzed biochemical and physical parameters.

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Oral presentation

Barley genotypes display differential physiological and molecular response to progressive water deficit

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We have analyzed the changes in physiological parameters (relative water content, RWC; biomass; water use efficiency, WUE; net photosynthetic yield, PN; quantum yield of PSII, Fv/Fm; proline and sugar content) in seedlings of nine barley genotypes subjected to a progressive increase in water deficit. Control plants were grown in 65% of soil water content (SWC) and experienced a period of water deficit when the SWC in each pot successively declined to 30%, 20% and 10% (severe drought conditions). Seedlings of all genotypes wilted when the SWC declined to 10%, but recovered turgor within a few hours of re-watering. In addition, when severe drought conditions were prolonged for a week, large differences in survival characteristics were observed between genotypes after re-watering. Multivariate analysis of the changes in physiological characteristics allowed to distinguish several different homogenous groups within the genotypes the composition of which was dependent of the stress intensity. Furthermore, integration between the stress-response traits was found and was shown to vary depending on the genotype and the stress level.
Expression profile of genes reported to be associated with the barley response to water deficit, including *LEA* genes, *NHX1*, *Hsdr4*, *BLT101* and genes encoding transcription factors (*HvDREB1*, *HvABF1*, *HVABI5* and *HvZIP1*) was analyzed in the seedlings of nine genotypes under progressive water deficit by semi-quantitative RT-PCR. The expression data reveled that increase in transcripts levels corresponding only to the *HvZIP1*, *Hsdr4* and *DREB1* genes was associated with better adaptation ability of seedlings to water deficit (de Mezer et al (2014). The changes in expression levels of the seven *Dhn* genes, *Dhn3*, *Dhn4*, *Dhn7*, *Dhn8* and *Dhn9* out of the eleven were substantial in all of the genotypes in response to progressive decrease in SWC, but the induction of their expression was rather associated with the level of RWC decrease in the plant tissues than adaptation ability to the stress conditions. Expression of the *HvZIP1*, *Hsdr4* and *DREB1* genes in response to water deficit was further confirmed at the protein level, and these genes may serve as expression markers for selection of barley genotypes with better adaptation ability to drought-prone environments.

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Oral presentation

Structural modifications induced by a drought/rewatering cycle in barley leaves

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Mechanisms of acclimation to stress may include modifications of body structure at whole plant, organ as well as tissue levels. Structural trait syndrome associated with tolerance of drought is known as xeromorphism. We searched for xeromorphic modifications in leaves of eight barley cultivars and two populations of SSD lines subjected to a drought/rewatering cycle under greenhouse conditions. Leaves initiated under drought were allowed to mature after rewatering and were then sampled for scanning electron microscope and light microscope studies of surface features and cross-section features, respectively.

Drought resulted in a reduction of leaf lamina length and width relative to well watered controls. Leaves from droughted plants had smaller epidermal pavement cells suggesting that inhibition of cellular growth was partly responsible for the lamina size reduction. Also stomata in droughted plants were shorter and narrower than in controls. Leaves formed under the influence of drought were thinner (both at the midrib and in intercostal regions) and had a thinner mesophyll, with only a slight reduction in epidermal cell thickness. There was thus no evidence for enlargement of epidermal bulliform cells, responsible for the leaf rolling response in grasses. Sclerenchyma was less well developed in droughted plants than in controls. The density of foliar trichomes was greater in droughted plants. Drought had no effect on the volume fraction of mesophyll and both epiderms in the whole lamina volume.

Traits characterizing water transfer efficiency (vein and xylem cross-sectional area as well as metaxylem vessel diameter) showed pronounced reductions under the influence of drought in proportion to reduction in lamina area. In contrast, vein density and stomatal density were enhanced by drought. Drought did not affect vessel wall thickness and the response of mesophyll cell wall thickness was genotype specific.

Significant allometric relationships between leaf lamina size and many anatomical traits computed across treatments and the diverse genotypes indicate that the reduction of leaf area might have driven majority of the anatomical alterations in barley leaves. Anatomical modifications usually occurred in proportion to leaf area reduction, however for individual traits (e.g. vessel diameter) in some data subsets the response to drought was relatively stronger, suggesting operation of an area-independent acclimative mechanism that modified leaf structure towards an improved drought tolerance. Interpretation of xeromorphic modifications in leaves subjected to a drought/rewatering cycle should, however, depend on water availability subsequently to the single drought episode. If drought returns, leaf xeromorphism will be advantageous, however under restoration of abundant soil moisture genotypes with xeromorphic leaves will likely be less productive than those with mesomorphic leaves.

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Poster 5.1

Free oxygen radicals and the in enzyme decomposition in barley leaves subjected to drought

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In plants, generation of oxygen radicals ($O_2^{\cdot-}$), the by-products of various metabolic pathways is often enhanced by environmental stresses, such as drought. The intensity of $O_2^{\cdot-}$ formation depends on plant genotype and its developmental stage. As the $O_2^{\cdot-}$ level is under control of the enzyme superoxide dismutase (SOD), the aim of the study was to compare $O_2^{\cdot-}$ generation and SOD activity in leaves of plants of 100 SSD lines of barley (*Hordeum vulgare* L.) subjected to drought in a pot experiment. Drought was applied at the seedling and the heading stage. The level of drought was 20% of field water capacity, whereas the control was 60%. Seedlings were collected after 8 days of stress, and the 4th leaf was taken, while mature plants were harvested after 14 days, and the leaf from a node under the flag leaf was used. The intensity of $O_2^{\cdot-}$ generation was analysed histochemically with nitroblue tetrazolium. SOD activity and protein content were assayed spectrophotometrically, by cytochrome *c* and Bradford method, respectively.

Plants of an individual SSD line responded differentially to stress, which was reflected in the pattern of changes in SOD activity, $O_2^{\cdot-}$generation and protein content, although it was dependent on the developmental stage of plants, too. In case of SOD activity in seedlings' leaves, the increases prevailed over the drops (40 versus 18), while the opposite trend was distinct in leaves of mature plants. The pattern of $O_2^{\cdot-}$ staining differed from marginal, through the patchy one to the complete coverage of a leaf. Based on the results, some genotypes were selected as potentially resistant to water scarcity occurring at the seedling stage (low $O_2^{\cdot-}$ generation and high SOD activity), whereas the others as extremely susceptible because of inhibited $O_2^{\cdot-}$ formation accompanied with extremely low SOD activity. As $O_2^{\cdot-}$ generation in mature plants can be greatly enhanced due to the accelerated drying during the spike formation period, "antioxidant phenotyping" fails in this case and can be applied as a breeding tool in case of seedlings only.

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Poster 5.2

Proteomic analysis of drought-responsive changes in roots of barley (*Hordeum vulgare L.*)

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Barley is one of four most important cereals in worldwide production. Drought is one of the greatest factor limiting plant growth and the productivity of crops. In response to water deficit plants developed various biochemical and physiological mechanisms. Factors, such as drought, cause significant changes in gene expression profile. Products of stress induced genes are classified as directly involved in tissue protection against dehydration and as proteins related with control of gene expression and signal transduction. Identification of this proteins is important for plant breeding programs and can improve a yield under drought conditions.
The aim of conducted research was the analysis of changes in barley proteome under drought stress. Changes in protein expression patterns in response to water deficit were monitored in four different genotypes of barley (Maresi and Cam/B1/CI, Stratus and Sebastian). Comparative analysis of proteins was studied by 2D PAGE and MALDI-TOF mass spectrometry. Proteins for qualitative and quantitative analysis were isolated by phenol extraction and separation by 2D gel electrophoresis. Obtained gels were analyzed in Image Master 2D Platinum software. Protein spots, which showed changes in expression profile, were analyzed by MALDI-TOF or MALDI-TOF/TOF mass spectrometer. The registered mass spectra (Peptide Mass Fingerprint) were compared with these from databases (MSDB, SwissProt, NCBI), using the MASCOT program.
Quantitative analysis of gels from roots sample separation revealed 335 total protein spots that significantly changed accumulation levels when samples from control and stressed plants were compared; and ~50% were identified by mass spectrometry. The highest number of root drought responsive proteins was observed in Cam (104). The proteome response in the three remaining genotypes was similar. We detected expression changes in 78 root proteins in Maresi, 75 in Sebastian, and 78 in Stratus. Root drought-responsive proteins were grouped based on their function in biological processes. Proteins exhibiting decreased expression levels were primarily involved in carbon metabolism. Proteins with increased accumulation were mainly associated with plant defense mechanisms. Many of identified proteins were general abiotic stress indicators (e.g. HSPs, ROS scavengers), others were related to the physiological processes severely affected by drought (e.g. carbon metabolism, nitrogen metabolism), and the remaining were involved in biological processes, which to date have not been recognized as directly associated with drought tolerance (e.g. phenylpropanaoid metabolism).

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