



WROCLAW UNIVERSITY  
OF ENVIRONMENTAL  
AND LIFE SCIENCES

  
**EUCARPIA**

European Association for Research on Plant Breeding  
Europäische Gesellschaft für Züchtungsforschung  
Association Européenne pour l'Amélioration des Plantes

# **International Conference on Rye Breeding and Genetics**



## **Conference Abstracts**

**Wrocław, Poland  
24th - 26th June, 2015**



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# International Conference on Rye Breeding and Genetics

Abstracts of Oral Presentations and Posters

24th - 26th June 2015 Wrocław, Poland

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## Preface

Dear colleagues,

the organizers are very glad to welcome you at Wrocław University of Environment and Life Sciences, Poland, for the International Conference on Rye Breeding and Genetics in the frame of the EUCARPIA - Cereals Section. After each of three conferences in Poland (2 x Poznan, Radzikow) and Germany (Schwerin, Stuttgart-Hohenheim, Groß Lüsewitz) and each of one conference in Sweden (Svalöv), Russia (St. Petersburg) and Belarus (Minsk) we are happy to be in Wrocław. Given the rather small group of rye breeders and scientists worldwide it is a great achievement that we meet here for the 10th Conference in this series. The impressive scientific programme comprises 26 lectures and 44 posters presented in six sessions. To combine science and practise a visit at the KWS LOCHOW POLSKA breeding station in Kondratowice is included. We want to thank all sponsors who helped with material or financial support.

Since our last conference in Minsk 2010 considerable changes occurred in rye research and breeding. The registration of the first commercial rye hybrid cultivars occurred meanwhile 30 years ago in Germany due to the work of Prof. Geiger and his group in Hohenheim. Since then, hybrid breeding was a success story gaining high interest in all rye-growing countries. The development of DArT markers firstly allowed us to do comprehensive QTL analyses with sufficiently dense maps for exploring the genetic architecture of quantitatively inherited traits. The development of the first 5K Rye Chip in a private-public partnership was surely a landmark opening the way for new technologies, including association mapping and genomic selection.

Still a lot has to be done to improve rye germplasm according to new breeding goals, including drought tolerance, nitrogen efficiency, and feeding quality combined with a grain yield that is competitive to that of triticale and wheat. To achieve these goals, it will be crucial to gain new genetic variation and to introduce it into elite germplasm. The close cooperation of rye research and rye breeding will be a clue for solving these challenges and to provide our crop a bright future.

The participants are happy that two distinguished rye researchers and Honorary Members attend this conference: Prof. Dr. Dr. h.c. H. H. Geiger (Hohenheim) and Prof. Dr. V.D. Kobyljanski (St. Petersburg).

Thomas Miedaner  
(Vice Chairman)

Henryk Bujak  
(Chairman)

Andreas Börner  
(Head EUCARPIA Cereals)

# **Program of International Conference on Rye Breeding and Genetics 24th - 26th June 2015, Wrocław, Poland**

## **Tuesday, 23th June 2015**

- From 16.00 Arrival, Hotels: Jan Paweł II, Wrocław, 2 Św. Idziego St. or DS Labirynt, ul. Sopocka 23 or DS Raj ul. Pautscha 5/7  
18.00 – 19.00 Participant registration (Jan Paweł II Hotel)  
19:00 Dinner & welcome meeting (Jan Paweł II Hotel)

## **Wednesday, 24th June 2015**

Conference at Wrocław University of Environmental and Life Sciences, 24A Pl. Grunwaldzki

- 8.30 – 9.00 Participant registration  
9:00 Addresses of Welcome  
Welcome by Wrocław University of Environmental and Life Sciences authorities  
Presentation of information about city of Wrocław  
9:30 Welcome of the head of EUCARPIA Cereal Section, A. Börner, Gatersleben, Germany  
9:40 Lucjan Madej: A personal reminiscence by J. Zimny, IHAR Radzikow, Poland

### **Session 1: Thirty years of hybrid rye cultivars**

Chair: H.H. Geiger, University of Hohenheim, Germany

- 9:50 T. Miedaner, University of Hohenheim, Germany  
From phenotype to genomics (and back) - Breeding research in the last three decades  
10:20 P. Wilde, KWS LOCHOW, Germany  
Genetic gain from hybrid rye breeding: achievements and challenges

**10:50 - 11:20 Coffee & Tea Break**

### **Session 2: General breeding methods**

Chair: P. Wilde, KWS LOCHOW, Germany

- 11:20 J. Larsen, Lethbridge Research Centre, Lethbridge, Canada  
Historical, current and potential future trends for rye production and breeding in Canada  
11:50 M. Pomomareva, Tatar Research Institute of Agriculture, Kazan, Russia  
A.A Goncharenko, Agricultural Research Institute of Central Region Non-Chernozem Zone, Nemchinovka, Russia  
Breeding progress and trends of winter rye yield in Central Russia  
12:15 Z. Banaszak, Danko, Poland  
Rye varieties in Poland  
12:35 E. Urban, I. Hardzei, SPCAF, Minsk, Belarus  
Breeding trends and cultivation of rye in Belarus

**13:00 - 14:00 Lunch at the University**

### **Session 3: Genomic prediction and selection**

Chair: M. Sorrells, Cornell University Ithaca, USA

- 14:00 M. Sorrells, Cornell University Ithaca, USA  
Genomic Selection in Plants: Training Population Design and GxE
- 14:30 M. Schönleben, TUM Munich, Germany  
Genomic prediction across breeding cycles in rye
- 15:00 K. Schmid, University of Hohenheim, Germany  
RYE-Select: Genomic differentiation and artificial selection in the two German rye (*Secale cereale*) breeding pools
- 15:20 Y. Wang, Univ. Hohenheim/IPK Gatersleben, Germany  
Genome-wide prediction of testcross performance and phenotypic stability for important agronomic and quality traits in elite hybrid rye
- 15:40 – 16:10 Coffee & Tea Break**

#### **Session 4: Emerging molecular tools**

Chair: P. Masojc, West Pomeranian University of Technology, Szczecin, Poland

- 16:10 E. Bauer, TUM, Freising, Germany  
Rye – Don't be afraid of an 8 gigabase genome jigsaw
- 16:40 B. Hackauf, Julius-Kühn-Institute, Groß Lüsewitz, Germany  
Approaching complex traits in the rye genome by association mapping and comparative genomics
- 17:10 V. Korzun, KWS LOCHOW, Germany  
Genomics and molecular breeding in rye - Current view and future development
- 17:30 P. Masojć, West Pomeranian University of Technology, Szczecin, Poland  
Bidirectional selective genotyping – a method to reveal molecular mechanisms underlying preharvest sprouting in rye
- 17:50 S. Stojalowski, West Pomeranian University of Technology, Szczecin, Poland  
Towards localization of restorer genes for the C source of cytoplasmic male sterility in rye
- 18:10 J. Zimny, IHAR Radzikow, Poland  
Androgenesis in rye - past and present
- 18:30 End of the first day
- 19:30 **Gala dinner**

#### **Thursday, 25th June 2015**

Conference at Wrocław University of Environmental and Life Sciences, 24A Pl. Grunwaldzki

#### **Session 5: Use of genetic resources and cytogenetics**

Chair: A. Börner, IPK Gatersleben, Germany

- 9:00 P. Dopierała, KWS LOCHOW, Poland  
Use of inbred lines from ancient Polish population varieties to broaden genetic diversity for hybrid rye breeding
- 9:20 A. Houben, IPK Gatersleben, Germany  
Origin and regulation of rye B chromosomes
- 9:50 A. Lukaszewski, Univ. California, Riverside, USA  
Introgressions from wheat to rye
- 10:20 E. Gacek, COBORU Słupia Wielka, Poland  
Legal bases and new trends in DUS testing in cereals
- 10:50 – 11:20 Coffee & Tea Break**

## **Session 6: Disease resistance and tolerance to abiotic stresses**

Chair: T. Miedaner, Univ. Hohenheim, Germany

- 11:20 K. Flath, Julius-Kühn-Institute, Kleinmachnow, Germany  
Pathosystem analyses of rye/leaf rust and stem rust populations
- 11:50 B. Fowler, Univ. Saskatchewan, Canada  
Breeding for frost tolerance in rye
- 12:20 M. Rakoczy-Trojanowska, Warsaw University of Life Sciences, Poland  
Association analysis of benzoxazinoid-related traits in rye
- 12:40 A. Gordillo, KWS LOCHOW, Germany  
Evaluation of backcross progenies followed by molecular breeding approaches to develop rye hybrids adapted to new environments
- 13:00 – 14:00 Lunch**  
**14:00 – 15:00 Poster session & discussion**

## **Session 7: Nutritional and technological quality**

Chair: H. Bujak, Wrocław University of Environment and Life Sciences, Poland

- 15:00 D. Boros, IHAR Radzikow, Poland  
European rye for enhanced food and feed
- 15:30 V. Kobylanski and O. Soloduchina, VIR, St. Petersburg Russia  
Rye breeding for low water soluble pentosans and possibility of its use in animal feeding
- 16:00 Closing Session (New location for next meeting in 2020 etc.)
- 16:30 – 18.30 Wrocław Sightseeing Tour**  
**19:00 Dinner & social meeting in a restaurant**

## **Friday, 26th June 2015**

A coach trip to the Breeding Station KWS LOCHOW POLSKA to Kondratowice

- 8:15 Meeting in the hotel lobby (Jan Paweł II Hotel)
- 8:30 – 9:30 Transfer to Kondratowice
- 9:30 – 10:15 Welcome in KWS LOCHOW POLSKA company  
Presentation KWS CEREALS activities in Poland- A. Szaśiadek, KWS LOCHOW, Poland  
Presentation of rye breeding work of KWS LOCHOW in Poland – P. Dopierała, KWS LOCHOW, Poland
- 10:15 – 10:45 Coffee & Tea Break**
- 10:45 – 11.15 Rye Belt activities presentation – C.H. Heuer, KWS LOCHOW, Germany
- 11:15 – 11:30 Presentation of strategic sponsor
- 11:30 – 13:00 Tour on the breeding fields and facilities in Kondratowice
- 13:00 – 14:00 Lunch in Kondratowice**
- From 14:00 Departure of participants**

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# **Oral Presentations**

(Ordered according to the program)

## **Thirty years of hybrid rye cultivars**

(Session 1)

# From phenotypes to genomics and back – breeding research in the last three decades

T. Miedaner

University of Hohenheim, State Plant Breeding Institute, Fruwirthstr. 21, 70599 Stuttgart, Germany, [miedaner@uni-hohenheim.de](mailto:miedaner@uni-hohenheim.de)

Thirty years ago, in 1985, the first three winter rye hybrid cultivars appeared in the Recommended List in Germany. They were bred by the University of Hohenheim in the group of Prof. Dr. H.H. Geiger in close cooperation with practical breeding companies. Hybrid cultivars increased their importance steadily and reached about 70% of the rye acreage in Germany ten years later. This proportion remains till today and 26 hybrid cultivars from three breeding companies are now released. They combine high grain or biomass yield with agronomic features (resistances to lodging, leaf rust, and ergot) and quality traits for baking, feeding and biogas production.

Breeding research was dominated in the beginning by genetic analyses of the hybrid mechanism and optimization of breeding plans on the basis of quantitative-genetic parameters that were derived from multi-environmental field tests. In a second phase, research on resistance genetics and selection for leaf and stem rust, foot rot, Fusarium head blight, and ergot were advanced. This included artificial infections with various methods in greenhouse and field environments with time- and resource-demanding phenotyping.

With the upcome of the first molecular markers suitable for rye in the 1990s, the analysis of pollen-fertility restoration became a main aspect of research. The availability of denser maps due to new marker types favored analyses of quantitatively inherited, complex agronomic traits. We analyzed two introgression libraries comprising in total 78 lines and several segregating mapping populations with  $\geq 220$  progenies with multi-environmental phenotyping and DArT, SSR, and SNP genotyping for up to eight traits, including grain and biomass yield, plant height, thousand-kernel weight, protein, starch, and pentosan contents under rainfed and irrigated conditions. Experiments were performed in cooperation with the companies KWS LOCHOW and HYBRO. For all traits, significant genotypic variation with moderate to high heritabilities was found in elite mapping populations and introgression libraries. Variation for pentosan content was restricted due to a former intensive selection for baking quality. Genetic correlations of grain yield between drought and rainfed conditions were narrow ( $r=0.9$ ), but low between grain and biomass yield ( $r=0.3$ ) under optimal conditions. Some cross-validated QTL, especially for thousand-kernel weight and quality traits, but also for grain yield, had major effects and were highly environmentally stable. For drought tolerance, specific QTL are already available in elite populations, but should be supplemented by additional loci. Genetic architecture of these traits and the efficiency of both population structures for detecting QTL will be compared. In the genomics era, phenotyping became more and more important and today large populations of about 1,000 genotypes are handled in several field locations. High-performance phenotyping based on automatic platforms with various sensor types might improve phenotyping and reduce costs.

These results provide good prospects for future genome-wide prediction in hybrid rye breeding. Final aim would be to phenotype only those genotypes with a high genomic breeding value. Testing should then be done in a factorial manner under water stress and optimal conditions to meet the future demands of winter rye growing.

# Genetic gain from hybrid rye breeding: achievements and challenges

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Compared to wheat or rice, rye is a minor cereal crop with a global acreage of about 5 millions/ha. In the course of 30 years of intensive investment into methodology, biotechnology and practical breeding hybrid cultivars gained a market share of about 20% of the total rye acreage. Actually the hybrid rye growing area is focused in Western, Central and Northern Europe. Optimistic perspectives for a further growth of the crop rely on excellent performance under low to medium fertile environments, and on high genetic gain from hybrid breeding.

Genetic progress is estimated from German national list trials (NLT) conducted in the years 2004-2014. Hybrid breeding achieves higher annual genetic gains (0.8 and 0.9 dt/ha, intensive and extensive agronomical level resp.) for grain yield compared to population breeding (0.3 and 0.4 dt/ha, intensive and extensive agronomical level, resp.). Yield progress is mainly driven by enhancing single ear weights. Significant trends are also visible for traits such as ear density and length of grain filling period, whereas no major progress is apparent for resistance to leaf diseases or lodging. A remarkable reduction of ergot susceptibility was achieved by the use of exotic genes for pollen fertility restoration. Yield losses due to disease breakdown plays a minor role due to buffering in the genetically heterogeneous four-way or topcross hybrids. Trends for on-farm yield were lower than those estimated in NLT possibly due to a shift from more fertile to less productive environments during the reference period.

In commercial hybrid rye breeding, population improvement is integrated into the first stages of parental line development. Crucial steps involved are (i) recombining pretested lines, (ii) inbreeding of their progeny, (iii) line per se test, (iv) testcross seed production and (v) test on general combining ability (GCA) to the opposite pool. On the long term, the breeder will benefit from population improvement with genetic gains accumulating from cycle to cycle by a proportional progress in variety development. Compared to schemes analyzed by Tomerius (2001) cycle length could be reduced from 6-9 to 5-6 years in actual breeding programs. Model calculations are presented suggesting that annual genetic gain can be improved by combining Phenotypically and Genotypically Estimated GCA (PEGCA and GEGCA, resp.) from testcross means and indices derived from genomic selection (GS) procedures if compared to purely PEGCA based selection. Accuracy (correlation of test to target criterion) of GEGCA should surpass  $r=0,4$  to allow for a robust superiority. The impact of combining PEGCA and GEGCA on dimensioning the five above mentioned technical steps is described under the assumption of a fixed breeding budget and various sets of variance components.

Subsequent to line development and population improvement, new hybrid candidates require a substantial developmental period before obtaining a product ready for commercialization. In the product development phase (PD), new lines have to be retested and ranked finally according to their agronomical value. Further, they are converted into

parental components meeting the demands for distinctness, uniformity and stability (DUS). In particular this refers to the cytoplasmic male sterile (CMS) A-line, the male fertile B-line and the CMS cross AxB on the seed parent side. On the pollen parent side, the best lines are intercrossed to form a restorer synthetic. Assessing genetic and morphological fingerprints of components and hybrids anticipates DUS results, and allows for retraceability in later seed production. PD ends with production of experimental hybrid and parental component seed for official tests. Approaches such as interim generations and marker assisted backcross procedures are discussed to reduce time demand for this phase from 3-4 (Tomerius 2001) to 2-3 years.

PD is followed by the certification process in official tests to assess VCU and DUS criteria of the hybrid candidates. In several European countries the certification process takes two years whereas in Germany three years of VCU testing are obligatory. Based on heritability estimates options are discussed to reduce the test period from three to two years. Farmers and breeding companies can benefit substantially from an official testing system combining data from abbreviated NLT procedures and subsequent regional advisory trials.

With seed production the commercialization of new hybrids starts. Compared to the beginning of hybrid breeding per se performance of inbred lines is 5-8 times higher due to an enormous genetic improvement and adapted agronomic techniques, thus reducing the developmental time lag.

Future development of hybrid rye will be pump-primed on four central action fields:

- i. To improve competitiveness, breeding methodology in rye has to keep pace with crops such as corn or wheat. Key technologies to be implemented in the next decade will be genomic selection, high throughput phenotyping, double haploid technique, and the exploitation of novel sources of genetic variation.
- ii. (Direction of use has to be broadened for food, feed, and bioenergy. In particular, feed use demands for customer-tailored products with enhanced feed value.
- iii. Climate change in general, and EU-wide regulations for nitrogen fertilizers in specific are coming up the horizon demanding for better water and nitrogen use efficiency, and changing competition among cereal crops.
- iv. Market area should be expanded from Western and Central Europe to the Eastern part of the continent. Canada and USA are also considered as developing markets.

# **General breeding methods**

(Session 2)

# Historical, current and potential future trends for rye production and breeding in Canada

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Historically, rye has played an important role in Canadian agricultural production systems, especially in the Prairie Provinces where it was a profitable crop that included additional benefits of effective weed control or being better suited to environmental conditions and soil types. Rye acreage has gradually declined over time to recent historic lows, however it is interesting to note that rye is still being grown in traditional rye growing areas and there is uptake of new rye varieties. At present, there is considerable optimism for rye production in Canada spurred on by expanded uses of rye in North America and the introduction of high yielding hybrid rye varieties to Canada. Presented will be historic data on rye production in Canada and a summary of rye varieties registered in Canada, including pedigree information for Canadian developed varieties. Current breeding goals for open pollinated rye at AAFC-Lethbridge include improving germplasm with regards to falling numbers, reducing incidence of ergot, reducing plant height, increasing grain yield, as well as improving biomass yield and biomass quality traits. Methods have been established to evaluate germplasm for sprouting resistance, enabling a recurrent selection breeding approach. Results will be presented from an ergot nursery based at Lacombe, AB which showed similar trends in ergot severity for check cultivars previously tested in the literature. These results indicate that this nursery should provide a useful tool to assist in the selection of varieties with reduced incidence of ergot. Perennial cereal rye is also being improved at AAFC-Lethbridge for both grain yield and biomass traits using cold tolerant and strongly biennial Canadian germplasm and perennial *Secale strictum* as parental lines. Agronomic evaluation of perennial cereal rye shows that it holds some promise as a dual purpose crop or for low input rye producers, provided it is managed properly.

## Breeding progress and trends of winter rye yield in Central Russia

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Tatarstan Republic is the largest region for the production of rye in Central Russia. Among the crops grown in the country, winter rye has a special place. It is well adapted to growing conditions and is characterized by relatively low requirements for soil nutrient elements. Compared to other crops, rye has several advantages, due to which with proper farming techniques yields her little fluctuate from year to year and often exceed the yields of spring cereals.

The first decade of this century, it was noted sharp decrease crop cultivation with 316 in 2001 to minimum 164 thousand ha in 2010. Reduction of the rye areas of this period was caused by the expansion of the winter wheat areas, very low prices and has ceased to be interesting and beneficial to agricultural farmers. In the past years, especially after the critical frost and drought 2010, the area under rye started to pick up.

The average yield of rye in Tatarstan in 1991-2010 equal to 2.67 t/ ha. For comparison, in the Russian Federation, the average yield for the same period amounted to 1.61 t / ha.

Presently, the breeding focus is again aimed at yield and resistance. In future, a progress in yield of more than approximately 1.5 % per annum is expected. Currently, the main task in the selection of rye should be to create varieties with good environmental adaptability, capable of generating the highest possible crop in a variety of growing conditions. Role of adaptive varieties increases with following factors environmental, climatic problems, less application of fertilizers and pesticides, increasing minimum soil treatment and brief period crop rotations, general decline in the level of technical equipment of agricultural production. Agroclimatic natural resources (temperature, moisture, and light energy) provide a range of changes in the yield in the region from 4.7 to 12.6 t / ha. The limiting factor is moisture. Potential rye provided by agroclimatic resources used on average by only 50%, and in favorable years – 68%.

This allows for utilizing favourable environmental conditions and also buffering unfavourable ones. Adaptive technological varieties of different directions used in conjunction with effective technologies of their growing importance, not only for the successful development of crop production, but also for sustainable livestock production. In recent years, the selection of winter rye is focused on targeted value. The main direction of selection of winter rye in Tatarstan is the creation of varieties of grain types, suitable for food and feed use.

## Rye varieties in Poland

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In Poland there are a lot of light soils. Only 3,5% of soils are of a very good quality, 63% medium and 22,5 of very weak quality. So rye is traditionally very important crop for farmers, because has low soil requirement and very good resistance to low temperatures and other stresses. All cereals' cultivated area is 7,2 million ha in 2013 and rye takes 17 % of that. Fifty years ago the acreage of rye in Poland was almost 5 million ha. In 2013 year – only 1,17 , whereas triticale area is 1,18 million ha, wheat – 2, 14, barley – 0,81, oats – 0,43. The average yield in production was in 2013 year 2,9 dt/ha for rye, 4,4 – wheat, 3,6 – triticale, 2,7 – oats and 3,5 for barley. In 1961 year all cereals yielded from 1,7 dt/ha in case of rye, 1,8 – oats and until 1,9 dt/ha for wheat and barley. At the end of 80s of the XX century the yield increase was caused by modern agronomical practices, while since 2004 was the result of an increase in crop varietal progress, including the introduction of hybrid varieties.

The number of tested rye varieties in the official trials in Poland (COBORU) was 16 in 2004 and 38 in 2014 year. In the meantime 7 – 20 population varieties have been tested and 9 – 18 F1 hybrids.

The Official Variety List contains 40 varieties (19 – populations, 18 – hybrids, 2 synthetics and 1 variety for green mass) in 2014 year. DANKO has 8 of them, KWS Lochoy – 7, Poznańska Hodowla Roslin (PHR) – 4, Hodowla Roślin Smolice (Smolice) – 6, Ramenda – 4, Saaten Union – 7, Monsanto – 3. Not all varieties have reproduction in Poland.

The total multiplication area of rye in 2004 - 2014 was 60757,8 ha and changed sinusoidal. The highest were noted in 2004, 2008, 2009, 2012 and 2013. Summarizing the multiplication area in those years the top area was occupied by population rye Dańkowskie Diament, followed by Dańkowskie Złote – very old variety listed in 1968 and hybrids: Visello, Palazzo and Brasetto. Taking into account the company market share in the years 2004 - 2014 the largest belonged to DANKO, followed by KWS Lochoy, PHR, Smolice and others.

The COBORU results of tested varieties indicated, that F1 hybrids yielded always better. The difference ranged from 9,8 dt/ha until 14,5 dt/ha on average (populations+F1) in 2004 - 2014 years. If we compared the top yielding populations and F1 hybrids the difference ranged from 10,6 to 16,0 dt/ha.

The brown rust resistance ranged from 5,6 to 7,5 on average in 2004 – 2014 years. The best note was 8,2. The mildew resistance ranged 6,3 – 8,3, the best variant was 8,7. Wider range was noted for lodging resistance from 4,9 to 7,7, the best note was 8,2.

The main use of rye in Poland is consumption, feed and industry. For consumption rye is very important, takes 19% of all consumption's cereals. Some part of rye is used for ecological farming, which is still growing.

It is interesting to indicate the importance of rye for triticales breeding. Not only octoploids are still produced, but also in the secondary genotypes rye is used. An example of great influence of rye for triticales is utilization of dominant dwarf gene Ddw1 for breeding new type of semidwarf triticales, which are very successful.

# Breeding trends and cultivation of rye in Belarus

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The basic cereals in Belarus are: spring barley (520 000 ha), spring wheat (190 000 ha), oat (150 000 ha), winter wheat (530 000 ha), winter triticale (520 000 ha), winter rye (330 000 ha). Such distribution of areas of the above-stated cereals is defined, first of all, by soil-climatic features of Republic, and also by the agricultural market. Areas of winter rye were reduced from 1 million ha (90-th years) to 330 thousand ha in 2014. It has occurred because of expansion of the areas of wheat, triticale and rapeseed. So, rye was replaced on less fertile soils. The given circumstance causes necessity of revision of methods and directions in rye breeding. At present the basic strategic directions of winter rye breeding in Belarus are following.

1. Increasing of productivity genetic potential: a) *Synthesis of varieties of populations with self-renewing intrapopulation heterosis*; b) *Development of F1 hybrids on the basis of CMS*.
2. Resistance to lodging: a) Improvement of architectonics and photosynthetic apparatus of a plant; b) Use of short-stalkness donors.
3. Increasing of adaptive potential: a) *Genetic resistance to environmental limiting factors (drought, high and low temperatures, etc.)*; b) *Early ripeness (shortening of interphase period length)*; c) *Sensitivity to fertilizers and protection means*.
4. Tolerance to main diseases: a) *Development of populations with monogenic resistance*; b) *Development of polyresistant populations*; c) *Development of populations combining race-specific and non-race specific resistance*.
5. Improvement of technological qualities of grain: a) *Development of varieties for purposeful use*; b) *bread making*; c) *production of mixed fodders*; d) *starch production and processing for ethanol*.
6. Development of varieties for green material: a) *High yield and nutritional value of green material*; b) *Ability to high growth after pasturing*.

Breeding methods, using for new varieties development are: screening of world winter rye diversity in the conditions of Belarus, experimental polyploidy, hybridization & stabilizing selection, development of heterotic F1 hybrids on the basis of CMS.

Now in Belarus 33 varieties of a winter rye are registered: 12 population tetraploid varieties, 14 population diploid varieties, 7 hybrid diploid varieties, from which 4 varieties are F1 hybrids of German breeding. Tetraploid rye occupies more than 50 % of areas under this crop in Belarus. Tetraploid varieties are used basically for the fodder purposes as are characterised by increased content of protein and low content of general and soluble pentosans in comparison with diploid varieties. Diploid varieties are mostly used for bread-making and alcohol due to high lodging resistance, low activity of  $\alpha$ -amilase ( FN 130-300 sec, amilogramme 450-600 A.U.), high specific kernel gravity (> 700 g/l) and increased starch content.

# **Genomic prediction and selection**

(Session 3)

# Genomic Selection in Plants: Training Population Design and GxE

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Inexpensive DNA sequencing and new statistical methods are facilitating new methods of crop improvement. Genomic selection (GS) is the simultaneous use of genome-wide markers to increase accuracy of performance prediction for both phenotyped and unphenotyped individuals in the breeding program. In GS, a training population related to the breeding germplasm is genotyped with genome-wide markers and phenotyped in the target population of environments. That data is used in a prediction model to estimate breeding values of selection candidates. Design of the training population is crucial to the accuracy of prediction models and can be affected by many factors including population structure and composition. Prediction models can incorporate performance over multiple environments and assess GxE effects to identify a highly predictive subset of environments. We have developed a methodology for unbalanced datasets using genome-wide marker effects to group environments and identify outlier environments. In addition, environmental covariates can be generated using a crop model and used in a GS model to predict GxE in unobserved environments and to predict performance in climate change scenarios. Current research is focused on optimizing the training population to improve efficiency and increase prediction accuracy in terms of genotypes, experimental design and environment sampling.

# Genomic Prediction across breeding cycles in rye

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Genomic prediction (GP) of breeding values and the subsequent selection and recombination of the most promising genotypes has become a standard tool for plant and animal breeders. Key feature of GP is the accurate prediction of genomic breeding values of yet unphenotyped individuals on the basis of their DNA profile. In plant breeding applications essentially two prediction scenarios arise - within and across breeding cycles. While substantial research has been dedicated to within cycle prediction scenarios, the main interest of the breeder lies in the prediction across breeding cycles, where GP has the potential to substantially increase response to selection due to a reduction of breeding cycle length and costs.

The objectives of our study were to i) investigate the prediction performance of genomic best linear unbiased prediction (GBLUP) within and across six interconnected cycles of a German hybrid rye breeding program using cross validation, and ii) evaluate the importance and effect of the kinship structure on the prediction performance of three economically important traits in rye. The study comprised data of 2,163 S2 inbred lines from the rye pollen parent pool, genotyped with 10,416 SNPs. Selection candidates were phenotyped as testcrosses for the traits grain dry matter yield (GDY), plant height (PHT) and thousand kernel weight (TKW) at multiple locations over six years (2009-2014).

Within breeding cycles, GBLUP yielded intermediate to high prediction accuracies for the traits GDY, PHT and TKW. In across cycle scenarios, we observed for all traits promising prediction accuracies on intermediate levels. Across cycle GP for cycles 2009 to 2012 yielded significantly ( $p < 0.05$ ) higher accuracies, compared to cycles 2013 and 2014, which can be attributed to the higher level of relatedness between the earlier cycles. For GDY we observe a linear relationship ( $r = 0.73$ ) between prediction accuracies and the across cycle maximum kinship. By augmenting the calibration sets with phenotypes of multiple cycles, predictions for all traits could be improved. To gain additional insights into the main components driving prediction performance, we will further analyze the across cycle prediction scenarios with respect to the effect of genotype by environment interactions.

# **RYE-Select: Genomic differentiation and artificial selection in the two German rye (*S. cereale*) breeding pools**

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Rye (*Secale cereale*) is a cereal crop that is gaining in importance in recent times due to its high levels of resistance to stress and frost tolerance. Due to its large, highly repetitive genome the genomic resources for rye were less developed compared to other crops. Together with the reference line Lo7, a total of twelve rye genotypes from the seed and pollen parent pools together with the *S. vavilovii* outgroup were resequenced. After stringent quality filtering over 10 million SNPs were left for the analysis of genetic diversity and to calculate the basic population genomic parameters of the breeding pools. Our focus was to find regions with high level of differentiation between the two pools, as those could contain pool-specific SNPs under positive selection during the breeding process. Regions with negative Tajima's D values in all individuals identify candidate selective sweeps acting on the whole breeding population. Additionally we used the software tools SweeD and OmegaPlus to scan the breeding pools for signatures of selection based on changes in allele frequencies. Both breeding pools exhibit a positive genome-wide average Tajima's D value (~0.2) and harbor numerous contigs with highly positive or negative Tajima's D values that either indicate strong selective or balancing selection and are interesting candidates for further validation. Signatures of potential selective sweeps were found in 144 pollen pool and 238 seed pool contigs. With the availability of the sequenced genome a 600k Affymetrix array has been produced and 136 individuals from a collection of wild lines and the two breeding pools were genotyped. Around 62,000 SNPs from that array come with an assigned cM position on the rye genome map. Using the LOSITAN software we found candidate SNPs for positive selection on each of the 7 rye chromosomes. Together with the whole genome data this will allow us to precisely identify genomic regions undergoing positive selection in one or both breeding pools, as well as map the level of differentiation across the whole genome.

# Genome-wide prediction of testcross performance and phenotypic stability for important agronomic and quality traits in elite hybrid rye

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Genomic (GS) and marker-assisted selection (MAS) provide powerful tools to accelerate the selection gain in plant breeding. Based on extended field evaluation data for grain yield, plant height, starch content and total pentosan content of elite hybrid rye derived from testcrosses involving two bi-parental populations that were genotyped with 1,048 molecular markers, we compared the accuracy of prediction of MAS and GS in a cross-validation approach. For all examined agronomic and quality traits, genomic selection outperformed marker-assisted selection. For yield stability, we observed absence of large effect QTL. In contrast, large and stable QTL were found for phenotypic stability of test weight, soluble pentosan content, and falling number. Applying GS that in contrast to MAS takes into account also loci with small effect sizes considerably again increased accuracies of prediction of phenotypic stability for all traits.

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# **Emerging molecular tools**

(Session 4)

## Rye - don't be afraid of an 8 gigabase genome Jigsaw

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For harnessing the full yield potential of rye, genome-based breeding strategies play an important role in increasing selection efficiency and accelerating breeding processes. Over the last years, we continuously advanced the molecular toolbox for rye, which now paves the way for a new era of genetics and genomics research and breeding in rye.

The rye genome is ~8 Gbp in size with a high proportion (>90%) of repetitive sequences. This has hampered whole genome sequencing (WGS) attempts in the past. Taking advantage of next generation sequencing we produced ~78x genome coverage of inbred line Lo7 using different sized libraries. From 46x coverage paired-end (PE) reads we generated a rye *de novo* assembly with a total sequence length of 1.7 Gbp which represents the non- or low repetitive portion of the genome. For scaffolding we used WGS contigs and mate pair sequences as well as in silico chromosome assignments which resulted in ~2.8 Gbp scaffolds with more than 66% of the represented base pairs assigned to specific chromosomes. Sequence annotation supported by RNA-Seq data and homology to other grass genomes delivered a set of around 28k high-confidence rye genes.

Additionally, we sequenced 10 diverse inbred lines and a *S. vavilovii* accession with medium coverage (~16x) to develop a high-density Affymetrix SNP array. SNP discovery was performed by mapping of PE reads of these 11 genotypes to the Lo7 assembly followed by variant calling. Comprehensive filtering of variants lead to a final number of 8.3M unique variants which were further filtered for the design of a 600k genotyping array. SNP performance was evaluated in a large panel of diverse samples from elite and exotic germplasm and in a mapping population. In total, 316,546 (52.7%) polymorphic SNPs were experimentally validated. A high-density genetic map comprising ~70k markers was generated which serves for genetic anchoring of the sequence contigs along the genome.

We used the Lo7 assembly as a reference to map RNASeq data of seven related grass species including *S. vavilovii*, *S. strictum*, and *S. sylvestre*, resulting in a total of 262,337 variants that give a first insight in the phylogenetic relationships of the selected species.

With this rye genome resource a reappraisal of syntenic and evolutionary relationships between Triticeae species and the analysis of structural genome variation will become

possible at an unprecedented level. The high density variant information is crucial for genome-wide screens to elucidate genomic regions differentiating the heterotic pools and/or carrying signatures of selection. A wealth of markers now is at hand for genome-based breeding and fine-mapping of genomic regions carrying traits of agronomic interest.

# Approaching complex traits in the rye genome by association mapping and comparative genomics

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Genetic improvement by artificial selection is known to cause a reduction in genetic diversity, which might be forced in the future by an implementation of the concept of genomic selection in hybrid rye breeding programs. Thus, the maintainance of genetic diversity is gaining increasing importance for the long term success of the genetic improvement of rye and asks for novel strategies to accumulate untapped beneficial QTL alleles from rye germplasm collections. An understanding of the genetic basis of various physiological, developmental and morphological traits provides novel options to improve yield, quality and sustainability of rye. Here we report on the results of a genome-wide association study based on a genotyping-by-sequencing approach and intensive phenotyping in a breeding population of hybrid rye. We have used DArTseq as a complexity reduction method to efficiently target 95,830 SNPs and 220,000 *in silico* DArTs in elite rye germplasm and integrated more than 16,000 markers covering 1,946 cM of the rye genome in the recently established transcript map of rye. We have estimated the average linkage disequilibrium to decay below the critical level within a map distance of 3-4 cM. A set of 320 experimental hybrids have been phenotyped in 2010, 2011 and 2012, respectively, for 12 agronomical and quality traits including grain yield. A novel, cross-validated NIR calibration allowed us to predict the content of crude protein, starch, water-extractable arabinoxylan and total arabinoxylan content in rye grains with unprecedented accuracy. The experimental hybrids revealed significant phenotypic variation for each of the assessed traits. All phenotypic data did not significantly deviate from normal distribution. The intensive phenotyping led to high heritability estimates for all traits including water-extractable arabinoxylan content. We have integrated significantly associated markers in an extended version of the recently published genome zipper of rye and identified genes, which are known to genetically control individual traits. The identified marker/trait associations provide a first step towards a targeted molecular characterization and utilization of genetic resources for precision breeding of hybrid rye varieties.

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# Genomics and molecular breeding in rye – current view and future development

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At the beginning of the 21st century, mankind faces the challenge of feeding a growing population with increasing demands of high quality food. Forecasts of climate change indicate additional uncertainty and place even more pressure on our natural resources to provide enough food for everyone.

In this context, genomics applied to rye and other crops, breeding programs play an important role in sustainably balancing the world's food supply and demand. Developing and production new varieties that are adapted to the different environmental conditions and make better use of available resources are crucial.

Conventional rye breeding is time-consuming and depends on environmental conditions. Therefore, breeders are extremely interested in new technologies that improve and optimise breeding processes. Molecular marker technologies offer a wide range of novel approaches to improve selection strategies in breeding. There has been a large and rapid accumulation of genomics tools in rye during the last decade. These developments have been coupled with the emergence of high throughput technologies, which have allowed advances in molecular marker analyses, implementation and evaluation.

The complete restoration of pollen fertility, corresponding to reduced infection by the ergot fungus, and increasing frost resistance to target environments in rye hybrids are prime targets in current practical breeding programmes. In all cases the breeding process can be accelerated by applying molecular markers for developing new material and for improvement of the selection intensity and accuracy.

The impact of new available molecular tools and technologies for plant variety development has proven to be essential to optimise and accelerate breeding programs.

The results of specific applications of molecular markers, potential of genomic selection and use of genomics in hybrid rye will be presented and discussed.

# **Bidirectional selective genotyping – a method to reveal molecular mechanisms underlying preharvest sprouting in rye**

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Bidirectional selective genotyping studies on preharvest sprouting and related traits carried out in biparental population of rye recombinant inbred lines (RILs) revealed genome-wide network of molecular markers and genes associated with extreme phenotypes. Several common loci and alleles showed different segregation patterns within groups of RILs representing extreme values for particular traits. Some alleles were associated with positive direction of selection for one trait and with negative direction of selection for another related trait. This result suggest that new validation procedures should be implemented before application of molecular markers in breeding. It is not possible to explain results of divergent selection shown in rye by an intra-locus mechanisms. New genetic mechanism controlling alleles response to divergent selection for quantitative traits is proposed.

## **Toward the localisation of restorer genes for the C source of cytoplasmic male sterility in rye**

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The C sterility inducing cytoplasm was discovered in the old cultivar Smolickie by prof. M. Łapiński. As it was evidenced by classic genetic analyses and molecular studies of mitochondrial DNA - the CMS-C is genetically different from the Pampa cytoplasm, which is commonly used in hybrid breeding of rye. Supposedly, the cytoplasm C is one of several sources of CMS reported by different authors, which can be classified as the Vavilovii (CMS-V) type of cytoplasmic male sterility.

Research related to identification of nuclear restorer genes for CMS-C was initiated at the beginning of 21<sup>st</sup> century. First experiments were performed on typical F2 populations developed from crosses between male sterile inbred lines and restorer lines. For construction of linkage groups mainly RAPD and ISSR markers were applied with addition of available SSRs and SCARs. Phenotypic analyses showed that control of male fertility restoration is of oligo-genic character. Application of interval mapping (IM) method has indicated the 4RL chromosome as the most significant for restoration of male fertility in rye with the C cytoplasm. Additionally, 2RL and 6RS chromosomes have been supposed to contain less efficient restorer genes.

As the precision of interval mapping was relatively low, for further studies more advanced mapping populations were developed: a Recombinant Inbred Lines (RIL) population originating from the cross between maintainer line with restorer inbred line and a BC5F2 population obtained after successive backcrossing: male fertile individuals selected from segregating back-cross generations were hybridized with male sterile maternal line (544-C). For analyze of both mapping populations new marker technologies were used, including DArT (Diversity Arrays Technology) and GBS (Genotyping by Sequencing). Application of efficient genotyping techniques for localizing of the main restorer gene Rfc1 allowed for identification a set of molecular markers closely linked to the gene.

Additionally there were obtained some preliminary results of comparative mapping aimed at study of relationships between Rfc1 and Rfp1 - effective restorer for the Pampa cytoplasm mapped on the 4RL.

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## **Androgenesis in rye- past and present**

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The success of heterosis breeding programs depends on the availability of a sufficiently large number of lines, which are genetically diverse. In this respect induction of doubled haploid lines from female and male breeding populations is quite important. The acceleration of production of breeding materials through androgenesis leads to genotypes with improved quality and productivity. Many years of experiments led to establishment of a methodology for production of DH rye (Immonen and Tenhola-Roininen 2003), but only for selected genotypes. This development does not mean that the method can be applied in other laboratories. The developed procedures for production of DH rye should work for a broader range of genotypes. It also appears that besides knowing the procedure, it is essential to have a long-term experience in the field of tissue culture. Studies on genetic determinants of the ability to regenerate rye in in vitro cultures are not advanced enough (Targońska et al. 2013). The results of studies conducted over the past decade helped to identify certain elements of the processes associated with in vitro regeneration. Currently, like in the past, it is crucial to develop an efficient method of plant regeneration. However, up to now, one of the unsolved problems is that the genotype still remains a major factor influencing the efficiency of plant regeneration from male cells of the gametophytic pathway. We intend to present current achievements in the field of production of doubled haploids of rye and to outline possible directions of research, leading to the use of rye androgenesis in breeding practice.

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Targońska, M., Hromada-Judycka, A., Bolibok-Bragoszewska, H., & Rakoczy-Trojanowska, M. (2013). The specificity and genetic background of the rye (*Secale cereale* L.) tissue culture response. *Plant Cell Reports*, 32(1), 1-9.

# **Use of genetic resources and cytogenetics**

(Session 5)

# Using inbred lines from ancient Polish population varieties to broaden genetic diversity in hybrid rye breeding

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Intensive selection in the process of hybrid rye breeding brings about a significant improvement of grain yield as well as of other agricultural traits within the parental pools. On the other hand, selection also narrows genetic diversity. Therefore sustainable breeding requires constant sourcing and inclusion of materials which broaden genetic diversity in the established parental pools. At the same time an appropriate genetic distance between the pollen and seed parent pools should be maintained. This demands a comprehensive analysis of the genetic resources (GR) before their incorporation into the appropriate parent pools.

In 2012, the Wrocław University of Environmental and Life sciences provided KWS LOCHOW POLSKA with a collection of genotypes from Central European rye populations. These collections consisted of inbred lines from ancient Polish varieties (over 70 years old), old Polish and Russian varieties (over 40 years old), as well as local populations. The GR lines were highly diverse, mostly exotic, and heterogeneous. Because they were developed before the era of hybrid breeding, it is highly probable that the lines contain valuable alleles which were lost in subsequent selection processes.

First, the GR lines were outcrossed to testers from the pollen and the seed parent pools to produce test cross seed. At the same time, homozygous sublines were obtained. Comprehensive laboratory analysis and climate-chamber tests were carried out for traits such as feeding quality, resistance to frost and snow mold, as well as to estimate the genetic distances and genetic structures using molecular markers. In the 2013/2014 vegetation season, the combining ability of GR lines was evaluated, based on multi-environmental field trials with their test crosses and on elite lines from both parent pools used as checks.

Based on phenotypic and genotypic estimates, 25 GR lines were selected: 10 were assigned to the pollen and 15 to the seed pool. The top GR lines reached 92 and 96% of the mean yield of the pollen and seed elite lines, respectively. Further GR lines excelled by huge variability in pentosan, protein and starch content recommending their use as donor genotypes in a feed breeding program.

Meanwhile BC1 progenies from selected GR lines with elite lines as recurrent parents have been produced to integrate them into appropriate parental pools.

# Origin and regulation of rye B chromosomes

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Supernumerary B chromosomes (Bs) are dispensable components of the genome exhibiting non-Mendelian inheritance and have been widely reported on over several thousand eukaryotes, but still remain an evolutionary mystery ever since their first discovery over a century ago. Recent advances in genome analysis significantly improved our knowledge on the origin and composition of Bs. In contrast to the prevalent view that Bs do not harbour genes recent analysis revealed that Bs of sequenced species are rich in gene-derived sequences. I will summarize the latest findings on supernumerary chromosomes with a special focus on the origin (1) DNA composition (2), gene erosion process (3) and the non-Mendelian accumulation mechanism (4) of rye Bs.

## Introgressions from wheat to rye

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Historically, various diploid rye species served as sources of intergeneric introgressions into cultivated wheat, both tetraploid and hexaploid. Perhaps the most famous of such introgressions is the 1RS.1BL chromosome translocation, originally selected from a triticale x wheat hybrid. It provided several loci for resistance to fungal wheat pathogens and insects, and now is present in hundreds of cultivars all over the world. Once the resistances broke down it became clear that the real benefit of the translocation is in increased root biomass. This affects grain yield, especially under water stress conditions. Many other introgressions from rye to wheat are also known. Reciprocal introgressions are almost non-existent. This is perhaps because chromosome manipulations in diploids are more difficult than in polyploids, or perhaps no one really tried. The first introgression from wheat to rye was in the form of added chromosome 5B in a diploid rye, produced by R. Schlegel, followed by monosomic and disomic substitution of 5B in a tetraploid rye, by this author. These served mostly in studies of the effect of the *Ph1* locus from wheat which controls chromosome pairing, and had no practical value. Contrary to expectations, even two doses of *Ph1* substituted into tetraploid rye did not make its meiosis diploid-like. The process of moving chromosomes and chromosome segments from wheat to rye is greatly facilitated by triticale, especially the tetraploids, so once chromosome manipulations involving the rye genome are done in triticale, it is quite tempting to transfer engineered chromosomes to rye. The first small segment introgression into diploid rye was chromosome 1R engineered in hexaploid triticale to improve its bread making quality. In this chromosome, a segment of the long arm with the *Sec-3* locus was replaced with a segment of wheat chromosome 1D with the *Glu-D1* locus encoding high molecular weight glutenins critical for good bread making quality in wheat. As in wheat and triticale, also in rye the locus significantly improved the parameters of bread making, but in its ca. 20-year existence it did not make it into commercial production. As the efforts to engineer chromosome 1R in wheat and triticale continued, eventually two new rye populations were created carrying not only the *Glu-D1* locus on 1RL, but also the chromosome-1 short arm loci: *Gli-B1/Glu-B3* and *Gli-D1/Glu-D3*. The former replaces the *Sec-1* locus of rye; the latter is in addition to *Sec-1*. These transfers were more challenging than *Glu-D1* and succeeded only in the third attempt, creating diploid ryes with only the *Sec-2* locus on chromosome 2RS left untouched. The consequences of this replacement will be illustrated on first small samples of grain. However, the effort illustrates that controlled introgressions from wheat to rye are feasible, thereby opening the wheat genepool for further exploration. It would appear that the effectiveness of wheat dwarfing genes in rye should be explored next, but any discussion of the priorities is welcome.

## Legal basis and new trends in rye DUS-testing in Poland

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Before the registration of new plant varieties in National Variety Catalogues, technical examination is performed by official bodies for variety's distinction, uniformity and stability (DUS) and for its value for cultivation and use (VCU).

The aim of DUS testing is to verify that the candidate variety is distinct from others, uniform in its characteristics and stable in the long run. The duration of the DUS examination of rye varieties is 2-3 years.

Once, the National Plant Variety Office (e.g. COBORU) considers that both DUS-tests and VCU assessment results are satisfactory and that all the other requirements have been fulfilled, the candidate variety can be officially registered in NL and/or plant breeder's rights can be granted.

Rye DUS-testing and examination criteria are traditionally based on the analysis of phenotypic data in standardized field trials. Traits with high heritability are used as the basis of DUS criteria for varieties. A rye – specific, DUS – testing procedure has been defined in UPOV (International Union for the Protection of New Varieties of Plants) Technical Guidelines (TG/58/6) and CPVO (Community Plant Variety Office) Technical Protocol (TP/058/1). These guidelines/protocol apply to open-pollinated varieties, but also to hybrids and their parental components.

Throughout the rye DUS-testing period, 22 different morphological/phenotypical characteristics are described in consecutive phenological plant stages, known to be optimal for the trait expression. These characteristics reflect the variety – specific morphology and physiology of plant organs, such as grain, stem, leaf and ear. For each trait, the method of assessment is defined either by actual measurement (M), by visual assessment by a single observation of a groups of plants or parts (VG) or visual assessment by observation of a number of single plants or plant parts (VS). The statistical analysis of the data assessed in the field trials are detailed in a UPOV Technical Working Party Document (TWC/18/10) resulting in the so-called combined – over years distinctness (COYD) and uniformity criteria (COYU).

Observed rapid development of marker and genome sequencing technology raises the chance to use molecular markers in DUS testing.

However, at the moment DUS decisions based on the DNA – based (molecular) markers, used alone in DUS testing, as a replacement of the assessment of phenotypic in the field are not yet officially considered to be fully acceptable.

It is widely expected, that UPOV will focus more on DNA – based technology for predicting phenotypic characteristics, only in the situation where molecular markers are fully predictive of the expressions of the phenotypic DUS characteristics defined in field tests.

# **Disease resistance and tolerance to abiotic stresses**

(Session 6)

# New resistances to leaf rust and stem rust in rye and the diversity of the respective rust populations in Germany

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Leaf rust caused by *Puccinia recondita* is a ubiquitous leaf disease in Central European winter rye. In contrast, stem rust caused by *P. tritici* f.sp. *secalis* is found mainly in areas with continental climates. Resistance breeding has been successful for leaf rust providing winter rye cultivars in Germany with disease severities from 3-7 on the 1-9 scale (1=healthy, 9=dead). Stem rust resistance, however, has not found enough attention till today. Our objectives were to (1) analyze leaf and stem rust populations for their diversity in terms of virulence phenotypes (=pathotypes), virulence complexity, and virulence frequency, (2) investigate the effectiveness of race-specific resistances in leaf rust and (3) search for resistance sources for stem rust in an international set of populations.

Leaf rust (2000-2002) and stem rust (2012-2014) populations were analysed on a national basis. In total, 827 leaf rust and 323 stem rust single-pustule isolates were tested with differential sets of 23 and 15 inbred lines, respectively. For both rusts, a high diversity of pathotypes was found with 317 (38% of total) and 226 (70%) pathotypes, the majority occurring only once. The three diversity indices (Shannon, Simpson, Evenness) were high in both rust populations.

To test the usefulness of race-specific leaf rust resistance genes in rye, synthetics were composed with 0 to 4 genes and field tested across 17 environments (location-year-combinations). Increasing host complexity and diversity, however, does not lead to a higher resistance level than that expected from the resistance of the parents. Only two synthetics with each of two resistance genes and two full-sib families of Russian origin stayed resistant across all environments.

For stem rust resistance, 14 genetic resources from Austria (Tiroler, Oberkärntner), Hungary (Hungarian Giant), Russia (Hy9a/86, Hy75/81, Hy2407/87, Talowskaja 29, Talowskija), USA (Wheeler, Elbon, Wrens Abruzzi, Gator, Alfa), and Argentina (Manfredi) with mean rust severities  $\leq 25\%$  (compared to 50% of susceptible standard) were identified as resistance sources. They contained 20-70% resistant single plants. Field resistance in adult-plant stage was well correlated with the resistance in leaf-segment tests in seedling stage.

In conclusion, the high diversity of pathotypes and the availability of highly virulent isolates should favor the adaptation of both rusts to simple, race-specific host resistances. More durable types of resistance might be achievable by combining the latter with adult-plant resistances.

# Breeding for Frost Tolerance in Rye

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The ability of a species to tolerate low winter temperatures is a primary factor determining its area of adaptation and distribution. Rye is the most frost tolerant (FT) of the winter cereal species and this advantage has allowed its production in a wide range of winter environments. However, complex genotype by environment interactions, its open-pollinated nature, and a high level of inbreeding depression often make rye variety development for specific winter environments a difficult challenge. A high level of FT is required for cultivars that must survive high stress winters like those in western Canada. As a result, cultivars developed for these regions must possess the right combinations of major and minor genes that maximize the FT levels found in rye. In addition to an ability to survive the extremes of winter, rye also has a genetic make-up that enables productive cool temperature growth and development, which is often as important as a high level FT for rye production in target areas with milder winters. Because the FT genes are down regulated once the plant receives the signal to enter the reproductive stage, the genes that are responsible for the rate of phenological development (vernalization, photoperiod, etc.) take on roles of greater importance and breeding programs for less winter severe environments acquire a whole new level of complexity. Conventional and molecular studies have identified chromosomal regions associated with FT tolerance and we now have molecular markers for a few major genes and a number of small effect candidates. These markers suggest that there is a role for marker assisted approaches in breeding programs for FT. More direct selection procedures, e.g., LT50, have also been utilized with success in selecting for FT. However, a large amount of unexplained heritability remains and there is still a need for field evaluation when selecting for cultivars specifically adapted to target regions. The importance of emphasizing these and related factors in rye breeding programs will be discussed in this presentation.

## Association analysis of benzoxazinoid-related traits in rye

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Benzoxazinoids (BX) are major secondary metabolites of gramineous plants that play an important role in disease resistance and allelopathy. They also have many other unique properties including anti-bacterial and anti-fungal activity, the ability to increase plant resistance to viruses transmitted by aphids and to reduce alfa-amylase activity in seeds. The aim of the presented study was to find associations between chosen traits potentially dependent on BX and genotype using Candidate Gene Association Mapping (CGAM) and Genome Wide Association Mapping (GWAM) approaches. In CGAM, 70 polymorphisms in the newly sequenced and characterized ScBx genes, and in GWAM – 5480 DArTSeq markers were applied. The following traits were included in the analysis comprising 152 Diverse Inbred Lines (DIL) derived from different polish scientific institutions and breeding companies: content of chosen BX (DIBOA, DIMBOA, HBOA, MBOA, GDIBOA, GDIMBOA) in above-ground parts and roots, resistance to brown rust and preharvest sprouting and activity of alfa-amylase in grain. Moreover, we were looking for SNPs associated with the expression level of ScBx genes. We have started our study with the determination of the DIL population internal structure upon DArTSeq genotyping results. Several groups could be distinguished on the dendrogram constructed using principal component analysis, however, only in case of DILs designated as W the correlation between clustering and seed origin was clearly visible.

In the majority of DILs, the heterozygosity level measured upon ScBx sequences and DArTSeq markers was lower than 10%. An exception were DILs designated as W where more than 25% of heterozygotic loci were observed in more than one third of lines.

CGAM and GWAM analyses allowed to detect a number of marker - trait associations, some exhibiting interaction with environment.

The identification of SNPs potentially associated with BX dependent traits can serve as a source of valuable markers for molecular breeding of rye.

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# Evaluation of backcross progenies followed by molecular breeding approaches to develop rye hybrids adapted to new environments

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The introgression of genetic resources (GRs) for the improvement of elite breeding populations is associated with low agronomic and yield performance from unadapted genetic resources. Per-se preselection (e.g. among S1 or BC1 plants) for simple traits (e.g. flowering time, standability) is an effective tool to ‘filter’ GRs. In hybrid systems, a strategy to introgress GRs in compliance with actual gene pools is essential. Different strategies can be considered to introgress GRs. Popular options are (i) phenotypic and/or genomic recurrent selection (RS) and (ii) introgression from advanced backcross (BC) populations or introgression libraries. RS may be connected with problems related to a suboptimal correspondence of the breeding material with elite gene pools, especially in the first RS cycle(s). Consequently, general combining ability tests may be biased and lines with high potential may be disregarded. Also, differences in the genetic structure (gene arrangements) between RS and elite populations can be expected. Therefore, the proportion lines from RS that can be integrated into the elite (second cycle) breeding programs should be limited to avoid disruption of genetic structure of elite populations. In case of the introgression from advanced BC populations, mapping populations are developed first, followed by the identification of recombinant inbred lines of interest. This strategy is associated with a large initial investment and limited sampling of gametes from very few GRs due to costs for development, phenotyping, and genotyping BC populations. Consequently, a small probability of identifying exceptional donors or unique chromosome segments is expected. Herein, an alternative strategy based on the systematic evaluation of large sets of BC progenies followed by marker-assisted selection is proposed. In specific, (i) large BC progenies (e.g. using different elite lines as recurrent parents) are produced, then (ii) new exceptional donors are identified, and (iii) targeted molecular mapping and breeding approaches are applied. In contrast to advanced BC mapping approaches, more focus is put on broad sampling and phenotyping a larger portion of GR variation. It involves a delayed but more targeted application of molecular approaches. More emphasis is put on the development of new ‘super-selects’. For ‘first-cycle’ introgression of GRs in a target gene pool, a BC1 pathway may be very effective. For established elite breeding programs, a more advanced BC pathway may be preferred to reduce the risk of disruption of elite gene arrangements and gene-pool structure compared to RS approaches.

# **Nutritional and technological quality**

(Session 7)

# European Rye for Enhanced Food and Feed

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Rye is an important cereal in Germany, Poland and Northern European countries. The grain of rye is used almost entirely by the domestic markets, with major distribution of its total production for food (35%), feed (44%) and industrial purposes (17%) (FAOSTAT, 2013).

As component of human diet rye has a long history, nevertheless the results of recent study have broadened our knowledge about its beneficial impact on our health and well-being (Poutanen, 2014). Rye is much better source of dietary fibre and other bioactive substances than wheat. Moreover, these substances in relatively large amounts are present not only in the bran fraction but also in the endosperm. The major DF components in rye are arabinoxylans, soluble and insoluble, having similar prohealthy effect as  $\beta$ -glucan in barley and oat and additionally they are decisive for its breadmaking quality. Rye contains also a whole range of phenolic compounds, which beside lignin include phenolic acids, phytates and alkylresorcinols. Phenolics with some other minor components belong to the group of so-called dietary fibre's co-passengers (Jones, 2010). In the epidemiological study it has been proved that rye DF and rye DF co-passengers stimulate weight loss, promote gastrointestinal health, lower the risk of type 2 diabetes, lower cholesterol level and improve cardiovascular conditions (Poutanen, 2014).

As component of animal feed, rye can provide a valuable source of energy and protein especially for pigs, but does have limitations because of lower palatability and high content of viscous arabinoxylans than in other cereals. Rye contains more digestible protein and total digestible nutrients than oat and barley but its feeding value is about 85 to 90% that of corn. The limitation of using rye for feed can be overcome by reducing their inclusion level and supplementing animal diets with enzymes that can hydrolyze arabinoxylans (Campbell and Bedford, 1992; Bedford, 1995; Boros et al., 1995).

It has been known that the content of nutrients as well as dietary fibre and other bioactive components in rye, similarly to other cereals, have varietal character, yet in a different extend modified by environmental conditions in which grain was produced. Taking all these information into account it is recommended to use varieties with the increased content of DF and DF co-passengers in quantities as large as possible for production of various foods. On the contrary, varieties with the reduced content of DF, especially soluble arabinoxylans, should be turned into feed industry.

In order to make better use of registered varieties of rye in Europe it is necessary to know the variability of their chemical composition. Such study was undertaken by team of SPOJPR at the IHAR-PIB. Overall 18 varieties of winter rye were characterized, produced in three contrasting agro-climatic conditions. The results revealed varieties the best for each uses.

## **Rye breeding for low water soluble pentosans and possibility of its use in animal feeding**

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Traditional rye grains surpass all cereals in nutritional and biological values; their protein corresponds to that of a cow milk at 83% whereas wheat protein corresponds only at 41%. But the use of rye grains for animal feeding is restricted by the presence of big quantities of water soluble pentosans (arabinose and xilose). Rye grains content of these substances is three times higher than that in grains of other cereals.

Water soluble arabinoxilanes in dry seeds are in polymeric state of the molecules but in animals stomachs form mucus restricting digestive enzymes access to grain nutrients. They are not hydrolyzed by animals' enzymes and are not fermented by yeasts allowing them to go through the entire gastrointestinal tract as mucus retaining their harmfulness.

Investigations on new directions in grain-fodder rye breeding have been conducted under the title "Development of rye with low content of water soluble pentosans in seeds" (2004-2011).

At searching initial material for breeding among 480 winter rye samples from world germplasm we have not revealed 'ready' low-pentosan varieties suitable for grain-forage use. But different rye populations were found to contain low-pentosan plants with frequencies 0,1–20%.

Five rye populations were identified containing approximately 20% genotypes with low contents (0,5-0,7%) of water soluble pentosans in grains. Decrease of the pericarp thickness up to 40-60% was found in low-pentosan seeds in comparisons with high-pentosan ones. This cause-effect relationship allowed us to formulate strategy and technology of low-pentosan rye breeding and seed-growing.

Together with breeders from other institutions we developed five varieties of grain-fodder winter rye that are under state trials. Seeds of low-pentosan forage rye do not form viscous mucus in animal stomachs. At their use as fodder for laboratory and farming animals high palatability and nutritional value were observed. To obtain the production increase (animal weight) at use of fodder unit the rye grains surpass all cereal crops. The use of low-pentosan rye seeds for forage purposes can decrease fodder conversion up to 42% in comparisons with patented mixed fodder.

# **Posters**

## **General breeding methods**

(Session 2)

# Characteristics of New Morphotypes Production Process of Winter Rye

poster s2-01

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The results of the research on winter wheat varieties developed at different time proved that the coefficient of the shoot weight usage for ear productivity formation and the degree of ear productivity potential achievement gradually increased. Thus, procurement of potential ear productivity by the shoot vegetative weight decreased. Therefore the increased ear potential cannot be completely satisfied both by “fresh” photosynthesis products and reutilization ones.

This is evidently exhibited during unfavorable weather conditions and leads to such undesirable effects as the decrease in the number of grains in the ear, weevil weight, steam strength, etc. Production process peculiarities of 14 morphotypes varied on the plants height, leaf size and orientation were studied to determine the types of plants with the greatest optimal architectonics. The excess of the investigated morphotypes over the evolutionary current ones on the shoot weight varied from 11.7 to 46.4 %, on potential productivity of the ear – from 15.4 to 34.0% and the real productivity of the ear – from 4.2 to 23.2%. Only two morphotypes used shoot weight more efficiently for the ear productivity formation.

The coefficient of usage in the rest ones was equal or lower (up to 18.8%) the standard. Morphotypes distinguished by the degree of potential ear productivity realization were differed by high potential ear productivity achievement, vegetative shoot weight and photosynthesis potential.

Common for the studied morphotypes is the fact that morphotypes different in intensive biomass accumulation by the phase of milky ripeness obtained low coefficient of its application. This negative correlation is a serious brake for the selective increase in winter wheat ear productivity.

# Phenotypic and genotypic evaluation of spring rye collection

poster s2-02

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In the agricultural experiences are mainly evaluated the yield and their quality parameters, but also other traits, including resistance to disease, pests, drought etc. In mostly cases statistical analyzes are performed separately for each trait. This approach does not allow for the implementation of an effective assessment covering the same time many of the features, often correlated with each other. Another problem is the large number of variables that hinders a comprehensive analysis of the results.

To detect the relationship between the characteristics assessed in the collection of 18 genotypes of spring rye - from different climate and soil conditions, was performed multivariate analysis of data. Principal component analysis was used as a standard method of multivariate analysis of the diversity of the objects in the collection. During three years experiment, which were established in two localizations such features as plant height, tillering term, the number of ears per 1 m<sup>2</sup>, number of productive ears, grain yield of 1m<sup>2</sup>, 1000 grain weight, hectolitre weight, total protein content, lysine and fiber were defined. In order grouping breeding material was used hierarchical cluster analysis. Calculations were performed using Statistica 10.0. The analysis of principal components allowed to assume that 78.77% of the total variation explained by three main components. The first component explained of 39.40% variability, and it was related to the total protein content (0.79), the content of lysine (0.86) and grain yield (-0.94). These features had the largest part in the multivariate variability in studied spring rye collection. The obtained results indicated that the genotypes with high protein content and lysine were characterized by lower yielding. The second main component explained of 23.64 % of the variance. The largest share of this component was the number of spikes ( $r = -0.72$  ). The third main component, which explained 17.74 % of the variation, was strongly connected with the thousand grain weight (0.73 ), and the time earing. Cluster analysis allowed the group the tested genotypes in order of their similarity in terms of all the ten studied traits. Primers used for genotyping ISSR UBC list # 9 . Generated products after using 30 primers were used to construction the genetic similarity dendrogram.

# Divergent selection on viscosity of a water extract at a winter rye

poster s2-03

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Results of divergent selection on a water extract viscosity (WEV) on the basis of varieties of a winter rye Alpha and Moscow 12 are presented. Relative viscosity of a water extract of a grain whole meal measured in santipoises (sP) on rotor viskosimeter VT5L/R by our technique. Efficiency of selection depend both on a genotype of a variety and from a direction of selection. Selection in plus-direction was more effective, than in a minus-direction. Asymmetrical the answer it was showed in 1-st cycle of selection and it was kept in the subsequent 8 cycles. Consecutive selection of offsprings high-viscosity plants has caused increase in potential of viscosity at a variety Alpha with 5,6 sP up to 17,4 sP, and at a variety Moscow 12 - with 6,7 sP up to 55,5 sP. After 9 cycles of selection divergent populations from a variety Alpha on the traits of WEV differed in 7,2 times, and populations from a variety Moscow 12 - in 12,3 times. At both varieties the curve of distribution of trait WEV deviated from normal and carried bimodal (two-peak) character. It is supposed, that the reason asymmetrical distributions is genetic asymmetry. The genes responsible for high potential WEV are dominant by the nature, have high primary frequency in a population and show strong additive effect. For this reason the success at plus-selection is achieved more quickly and more strongly, than at a minus-selection. The attribute of low viscosity, on the contrary, is determined recessive alleles with their rather low frequency in an initial population and weaker additive effect. Therefore the reason asymmetrical distributions of trait WEV is initial asymmetry of frequencies of all genes influencing potential of an attribute of viscosity in an initial population. Divergent selection on trait WEV correlative has changed many attributes of quality of grain. High viscosity populations had the best baking quality of grain, than low viscosity. Their superiority was showed in higher grain unit (on 4,9 %), mass of 1000 grains (on 14,8 %), number of falling (on 90 s), peak of amylogramm (on 328 u.a.), temperature of pasting (on 2,5°C). Low viscosity populations gave strongly blurring bread with large-porous, a sticky and restive crumb. It is shown, that at selection of a rye on improvement of baking quality direct selection on high WEV is more preferable, than selection on high falling number. The conclusion is made, that a method divergent selection on WEV it is possible to change purposefully quality of grain of a rye according to tasks of selection.

Keywords: winter rye, divergent selection, water extract viscosity, baking quality.

# Effective scheme for selection of genotypes with dominant gene of short stem in the breeding of winter tetraploid rye

poster s2-04

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Since 2000 till 2014 the rate of tetraploid varieties with dominant gene of short stem *Hl* was about 50% in total rye production in Belarus. The presence of long-stem plants of *hl4* genotype is a common problem in such varieties. It impedes their patenting and requires additional manual labour for uniformity control during seed production. The problem mentioned is a consequence of the presence of some proportion of allele *hl* in population. It appears because of impossibility to differ genotypes *Hl4* and *Hl3hl* by their progenies analysis using traditional method of “reserves”. V.D. Kobylanskiy had explained theoretical basis of homozygotation of tetraploid populations by *Hl* allele using two cycles of pair and analyzing crosses; E.P. Urban implemented it in practical breeding. However, this method needs substantial work for artificial isolation of plants; it's complicate to integrate it into common breeding process. Our estimation showed theoretical possibility to differ initial genotypes *Hl4* and *Hl3hl* using calculation of proportion of long-stem plants (*hl4*) in the 2nd generation (F2) of initial genotypes after open pollination of the progenies, representing their 1st generation (F1). The improved scheme of *Hl4* genotypes selection was developed. It included 2-year reservation of the part of seeds of elite plants as well as calculation of long-stem plants *hl4* proportion in the progenies in the breeding nursery (F1) and in the nursery of breeding progenies testing (F2). Homozygous by *Hl* allele population is formed using reserves of elite plants, which F2 progenies have proportion of long-stem plants *hl4* corresponding theoretically estimated value for F2 progeny of initial genotype *Hl4*. The scheme was used for homozygotation of 2 breeding populations in 2003–2007. One cycle of selection according to the scheme resulted in formation of populations, which were uniform in plant height. Economically valuable traits were improved simultaneously: grain weight of main spike increased on 12%, its fertility – on 2%, 1000 kernels weight – on 7%, crop yield – on 7%. Both populations were tested in the State trials; one was included into Belarusian registry of varieties with the name *Zazerskaya 3*. The scheme described can be used for improvement of tetraploid populations, which have proportion 0.1–3% of long-stem plants, as well as registered varieties in the process of their original seed production.

# Evaluation of the ability to androgenesis in Polish rye (*Secale cereale* L.) breeding materials

poster s2-05

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Double rye haploids (DH) (*Secale cereale* L.) are a potential tool for basic research and plant breeding. Methods of obtaining rye haploids include androgenesis (anther cultures and isolated microspore cultures) and ovule cultures or haploid embryo cultures after pollination with maize pollen. The effectiveness of rye haploidisation strongly depends on the genotype and requires basic research on the genetic conditions for androgenetic capacity for a broad spectrum of genotypes and it requires a methodology of effective DH line induction.

The aim of the study was to determine selected factors which are decisive to the effectiveness of plant regeneration in anther cultures of Polish rye breeding materials.

During the research we analysed the androgenetic capacity in entrusted breeding material (30 rye genotypes). The growth of donor plants for anther cultures took place in the experimental garden of the Department of Plant Breeding and Genetics, Poznań University of Life Sciences, Poland. When spikes with microspores at a uninucleate stage reached an appropriate stage of development, they were harvested, placed in distilled water and treated with a temperature of 40°C. The stage of development of microspores was checked by means of preparations stained with the acetocarmine squash method. Harvested spikes were kept in beakers with distilled water in cold rooms. The mean time of thermal treatment for the genotypes from PHR was 16.9 days, whereas the lines from DANKO were treated for 22.2 days on average.

The surfaces of randomly selected inflorescences of the plants under study with microspores at a late uninucleate stage were disinfected with 70% ethanol and 6% sodium hypochlorite (NaClO). Next, they were rinsed in large amounts of sterile distilled water. Anthers were isolated from the spikes and placed on Petri dish (90 mm in diameter) with 25 ml of medium C17 with 2 mg/l 2,4-D added, with C17 and 1 mg/l 2,4-D and 1mg/l of dicamba added (Wang & Chen, 1983) and with 190-2 with 2 mg/l 2,4-D added (Xingzhi & Han, 1984) to induce androgenesis. Anthers from one spike were placed in each pan. Anthers from 15 spikes were placed for each of the genotypes under study. The anther cultures were stored in darkness at temperatures of 26-28°C for 8-12 weeks in order to induce androgenesis. After eight weeks of storage the callus structures obtained from the anther cultures were transferred to a regeneration medium MS (Murashige & Skoog, 1962). The calluses were incubated at a temperature of 26°C and 16-hour photoperiod. Every 21 days they were transferred to a new regeneration medium.

During the experiment 49,402 anthers were placed, including 23,426 anthers from the collection of breeding materials of PHR and 25,976 anthers from DANKO genotypes. Callus induction was observed in all of the 30 genotypes tested. The effectiveness of callus formation was the highest in the anthers from PHR3 genotypes (147 calluses), PHR1 genotypes (63 calluses) and PHR5 genotypes (49 calluses) as well as in 572/12 genotypes (176 calluses) and S1194/12 genotypes (119 calluses). Anther cultures were started on three

mediums inducing androgenesis (C17 + 2,4-D, C17 + 2,4-D + dicamba, 190-2 + 2,4-D). As far as PHR genotypes are concerned, the induction of androgenesis was the most effective on medium 190-2 + 2,4-D – 256 calluses of 536 calluses obtained, on medium C17 + 2,4-D – 139 calluses of 536 calluses obtained and on C17 + 2,4-D + dicamba – 141 calluses of 536 calluses obtained. As far as Danko HR genotypes are concerned, we did not observe such considerable differences between androgenesis-inducing mediums. The numbers of calluses obtained were as follows: medium C17 + 2,4-D – 454 calluses of 1327 calluses obtained, C17 + 2,4-D + dicamba – 453 calluses of 1327 calluses obtained, 190-2 + 2,4-D – 420 calluses of 1327 calluses obtained. The research revealed that the genotypes investigated in the anther cultures were characterised by diversified androgenetic capacity. They ranged from forms with very low effectiveness of androgenesis induction (PHR11, PHR12, S1150/12, S1157/12) to forms which responded with the formation of large numbers of calluses and embryo-like structures (ELS) (PHR3, PHR1, 572/12, S1194/12).

We obtained 74 green regenerants for 9 of 30 rye genotypes tested (47.14% of regenerants). Green plants were obtained from one genotype from Poznańska Hodowla Roślin – PHR5 (2 plants). As far as the genotypes from Danko HR are concerned, we observed regeneration of green plants in 8 of 15 genotypes tested. The greatest number of plants was obtained for S1188/12 – 26 plants and for 572/12 – 19 plants. The greatest number of green plants was regenerated from callus structures obtained on the medium C17 with 2,4-D and dicamba added. The ploidy level of the regenerants was determined with a flow cytometer. The findings were as follows: 1C (haploids) – 18.75% of the regenerants, 2C (diploids) – 76.56% of the regenerants, 4C (tetraploids) – 4.69% of the regenerants.

The research findings revealed that that the genotypes under investigation exhibited different reactions to the mediums inducing the formation of microspore callus in anther cultures. The medium 190-2 with 2,4-D added was the most effective for the plants from PHR, whereas the medium C17 with 2,4-D and dicamba added was the most effective for Danko genotypes.

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# **Male fertility of hybrids between the two CMS variants of the inbred line 541 pollinated by different populations of rye**

poster s2-06

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Hybrid cultivars of rye have been grown for over 30 years. Almost all registered hybrids are bred with the use of the Pampa cytoplasmic male sterility (CMS-P) which was discovered by Geiger and Schnell (1970) in primitive rye population originating from Argentina. An alternative for CMS-P is the Vavilovii (CMS-V) type of sterility-inducing cytoplasm. This type of CMS was discovered by numerous authors within different populations of rye and it is represented by sources designed as CMS-G (currently present in some registered cultivars), CMS-C (studies in this research) and others. Study of different rye populations proved that CMS-V occurs commonly in cultivars distributed worldwide, especially in Europe. On the other hand, the CMS-Pampa is not widely spread and it has been detected mainly in populations from South America and incidentally from Near East of Asia. It is also known that in European rye populations restorer genotypes for the CMS-P are rare for breeding purposes it was necessary to introduce effective restorer genes from exotic plant materials. On the opposite side - it is easy to find in rye populations restorers for CMS-V but efficient maintainers are seldom. The aim of this study was to analyze Polish cultivars with respect to their efficiency in male fertility restoration/maintenance of sterility in CMS-P and CMS-V. Recent results from analyzes of Polish cultivars were compared to formerly collected data concerning 5 populations from Iran (Altevogt 14159, Altevogt 14160, Altevogt 14161, IRAN I, IRAN IX) and 3 from South America (San Jose, Pico Massaux, Trenelense) which were kindly provided for study by professors H.H. Geiger and T. Miedaner (University of Hohenheim, Germany).

For test-crosses two cytoplasmic male sterile versions of the 541 inbred line were applied (both after at least 10 cycles of back-crossing). Seeds were obtained from isolated top-crosses (separate for each studied population) and planted individually on the experimental field in Szczecin (at least 100 plants of each cross combination were analyzed). Assessment of male fertility was done visually with the use of the 9-step scale of Geiger and Morgenstern (1975). Significant differences in ability to restoration of pollen fertility were noticed dependent on the type of cytoplasm and origin of studied populations.

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# **Change of productivity, its elements and morpho-biological characters of the winter rye in the process of breeding**

poster s2-07

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To reveal the tendencies of winter wheat characteristics in the process of selection varieties developed during 40 years were studied. The investigation of 5 varieties during 3 years was conducted in Voronezh region in the experiment set by the scheme of the Latin triangular. The predecessor was clean fallow, the soil was chernozem layer of medium power and medium humus weather conditions were different in different years. Calculations and observations were carried out according to the Instruction Methods of State Crops Variety Testing. The indices characterizing the features of the productive process in course of grain formation were identified by the methods introduced by the staff of RSAA. The results of the research proved 1.5 increases in winter wheat yield due to selection. It was the result of the sowing specific density and the harvest index value. The increase of the varieties lodging resistance up to 31.4% was due to the decrease in the plant height and stem strength. As a result of selection close positive link between the shoot length and its productivity was damaged. Neglecting the length decrease relation between the ear and its elements to the shoot length gradually increased. Leaf index slightly increased as a result of the selection but the upper level leaves share increased by 50.9% Chlorophyll index at milk stage increased by 33.3%.

The ratio of the shoot mass usage on the ear productive capacity increased almost by half. The degree of its productive potential increased only by 13.5%. The reason is decrease of the vegetative part of the ear.

Thus winter wheat breeding should be directed to the shoot vegetative part increase primarily due to the leaf area increase.

# **Genomic prediction and selection**

(Session 3)

# Genomic selection in hybrid maize and rye breeding

poster s3-01

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In line (product) development, genomic selection (GS) schemes can be classified according to the relationship between the training population (TP) and prediction population (PredP) (same or different selection populations). GS schemes implemented in AgReliant Genetics' maize breeding program and KWS LOCHOW's rye breeding programs exemplify contrasting types of PredPs and different relationships between TP and PredP. In the hybrid maize GS scheme, large numbers of doubled haploid lines are developed within biparental populations. A portion of the lines is used as TP to predict untested lines from the same biparental population. Multi-environment validation experiments showed that prediction ability for grain yield is somewhat higher for lines in the TP and slightly lower for untested lines compared to phenotypic selection. Index selection using phenotypic and genomic grain yield values was consistently better than phenotypic or genomic selection alone (Krchov et al., 2015). Another advantage of this GS scheme is the possibility to predict lines with insufficient seed set that otherwise would be delayed in their testcross evaluation by one year, which saves time and simplifies logistics. In the hybrid rye GS scheme, the TP includes independent populations tested in previous years. Validation experiments indicated that the prediction ability for grain yield was considerably (> 200%) higher for phenotypic selection than for GS when predicted lines were not included in the TP and GS was equivalent to phenotypic selection when predicted lines were included in the TP. This indicates a strong influence of the genetic background on estimates of marker-allele effects. Index selection using phenotypic and genomic grain yield values leads consistently to (around 9%) higher prediction abilities than phenotypic or genomic selection alone. Predictions based on TPs from previous selection cycles indicate a drop in prediction ability in the years 2013 and 2014, probably due to a reduction in the genetic relationship between TP and PredP following a change in the genetic structure of the breeding populations. Validation experiments have shown that prediction abilities across selection cycles depend on the proportion of parents and uncles of predicted lines in the TP and can be increased considerably (> 39 %) when all parents are included in the TP. *Per-se* selection and variation in agronomic traits indirectly influencing yield may considerably affect prediction ability. Using data from the evaluation of lines in multiple years and appropriate modelling SNP by year effects as well as including all parents of the PredP in the TP may be key to increase prediction abilities of GS across selection cycles.

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# DNA markers in selection of restorer forms with CMS Pampa in rye

poster s3-02

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The cytoplasmic male sterility (CMS) phenomenon is observed in about 150 plant species (Kaul, 1988) from different taxonomic groups. This trait is commonly used in plant breeding. In the case of rye (*Secale cereale* L.), one of the most used systems is CMS Pampa (Geiger, Schnell, 1970). The sterilizing activity of Pampa cytoplasm is conditioned by the incompatibility of nuclear and cytoplasmic genomes. The main problem in CMS Pampa system is pollen fertility restoration. To restore fertility, nuclear genes with different strength are necessary. They are located in almost all of rye chromosomes, but the strongest are genes in 1R and 4R chromosomes (Miedaner, Glass et al. 2000).

Over past few years, the DArT markers associated with genes of pollen fertility restoration in CMS Pampa rye linked to the genes located on 1R, 4R, etc. (unpublished) were developed. The most promising ones are those mapped to the chromosome 4R as that gene is responsible for most of the phenotypic variance of the trait. Some of the DArTs linked to the 4R were converted to the PCR specific ones and tested on a broad range of breeding materials. They proved to be useful for backcrossing programs.

A set of seven parental lines with CMS Pampa but without the Rf gene on 4R were backcrossed to the donor line with that gene. The presence of pollen fertility restoration gene in every backcross generation was verified by PCR specific markers. Additionally, the presence or absence of the 1R Rf gene was confirmed using DArT based PCR markers. Several rounds of marker-assisted backcrossing allowed the evaluation of parental lines (BC3F1) with different composition of restore genes in distinct genotypes that are being tested for their combining ability.

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# Combining ability of genotypes in the breeding program of rye hybrids

poster s3-03

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General combining ability (GCA) of several female and male components for rye hybrids and specific combining ability (SCA) of parental pairs were determined. The parental components were taken from the current hybrid rye breeding programs of three plant breeding companies: Danko Plant Breeding Ltd., Poznan Plant Breeders Ltd. and Smolice Plant Breeding Ltd. The F1 progenies were derived by crossing several female components to few male components in a factorial mating design. The F1 hybrids seed were produced in spatial isolation fields. The experiments were conducted in the season 2012/2013 at three locations with three replicates (plot size - 5m<sup>2</sup> sowing density - 250 viable kernels/m<sup>2</sup>). The experimental design was a 6×6 lattice. The F1 hybrids were tested for agronomic traits, such as grain yield, heading (the number of days from 1.01), pollen shedding, plant height, lodging, powdery mildew resistance, brown rust resistance and 1000 grain weight. An analysis of variance was performed for each of the investigated traits combined across locations by using the Sergen 4 computer package (Caliński et al. 2003).

Considerable environmental effects and significant variation ( $P \leq 0.01$ ) among the F1 hybrids for all of the traits in the experiments were found. Hybrid × environment interactions were significant for majority traits. Variation due to GCA × environment interaction was more important than SCA × environment interaction. General combining ability of both females and males was significant for majority traits in the experiments. Significant specific combining ability was detected mainly for pollen shedding, plant height and 1000 grain weight. Moreover, SCA variance also was significant in one set of genotypes for grain yield, heading and brown rust resistance. GCA variance was more important than SCA for all the traits under study, indicating the importance of additive genetic effects. Non-additive genetic effects were significant mainly in determining of pollen shedding, plant height and 1000 grain weight. The tests allowed to select female and male components with significant favourable GCA effects for utilization in rye hybrid breeding.

Key words: combining ability, hybrid breeding, quantitative inheritance, rye, *Secale cereale* L.

# Identification of pollen fertility restoration markers in rye with CMS Pampa

poster s3-04

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The cytoplasmic male sterility (CMS) phenomenon in plants is based on incompatibility of nuclear and cytoplasmic genomes and results in the lack of production of functional pollen. Its implementation into breeding systems of cereals resulted in the development of hybrids of commercial importance. A good example is the exploitation of heterosis in hybrid rye with CMS-Pampa (Geiger, Schnell 1970). The evaluation of new hybrids in rye requires efficient parental lines that can restore pollen fertility. Pollen fertility is expressed by numerous genes (Miedaner, Glass et al. 2000), however the one located on the chromosome 4R is considered as the most important as it explains most of the phenotypic variance of the trait. Thus, its introduction into parental forms that originated from European material and lacked the gene is highly significant. This could be achieved if the appropriate markers are available.

RIL mapping population (maintainer (N) x restorer line with cms P based on Iranian materials) consisting of 92 F5/F6 individuals was genotyped with DArTseq markers. Pollen fertility restoration of the RILs was verified via crossing maternal plant (analogues of the maintainer line) on cms pampa with each RIL using visual scale (Geiger, Morgenstern 1975). Genetic map based on DArTseq markers was constructed under MultiPoint UltraDense software (<http://www.multiqtl.com>). Association mapping was performed in TASSEL (Bradbury et al. 2007). Cluster analysis was performed in PAST (Hammer, Harper et al. 2001). The genetic map consisted of 8 linkage groups covering 1516.43 cM. Out of 14 222 markers segregating in the population, only 790 were mapped with a few gaps spanning over 21 cM. DArTseq markers formed numerous groups of redundancy. Composite interval mapping allowed the identification of a QTL (spanning over 3cM) with LOD function maximum equal to 5.9 within LG1 (covered 267.06 cM) explaining over 25% of the trait variance. The marker closest to the QTL was 0.6 cM from its maximum. Additional markers were evaluated via association mapping (based on all available markers for the mapping population) and cluster analysis. Association mapping allowed the identification of markers (DArTseq & SilicoDArT) that were associated with the trait. There were 21 markers that passed Bonferroni test with association higher than 0.24. Based on cluster analysis, markers within the same group where the markers linked to the QTL were found, additional markers were chosen for the reconstruction of the linkage group with the QTL. The group contained 147 skeleton (plus 284 redundant ones) and covered nearly 250 cM. Composite interval mapping located the QTL between 221 and 240 cM. Its maximum was at 235 cM (LOD=4.3) and explaining nearly 20% of phenotypic variance. The closest marker was 0.15 cM from its maximum. There were 19 skeleton with 87 redundant markers within QTL. Currently, the affords are focused on assigning the LG1 to the rye chromosome.

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# **Molecular–genetic markers in winter rye (*Secale cereale* L.) for heterosis**

poster s3-05

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Due to cross-pollination and self-incompatibility, biological properties of rye favor development of hybrid varieties. Development of a constant line material and selection of crossing components are the basis of a high heterotic effect of winter rye hybrid F1. Use of conduct controlled crosses in rye without manual emasculation. A classical method for cytoplasm identification requires analysis of 2-3 hybrid generation. Molecular-genetic research methods shorten the period of identifying mitochondrial DNA (mtDNA) of male sterile (S) and normal (N) plants for different CMS types. Use of molecular markers (coxI, nad6, nad2) for mtDNA, described by Stojalowski et al. (2006), has allowed identification of male sterile (MS) forms based on CMS of P-, G-types and self-fertile (SF) lines of winter rye. Twenty four MS-lines were selected on the basis of G-type CMS, 44 MS-lines were determined on the basis of P-type CMS. The nad2 marker detected the fragment typical of mitochondrial DNA sequence with G-type cytoplasm in five SF-lines and in two sterility fixers with N-cytoplasm. PCR assay of nuclear DNA of the examined CMS genetic systems of P-, G-types and SF-lines of rye was made with three pairs of primers (SCY03, SCP16M58, SCP44M51) linked with fertility restoration genes (Rf) (Strake et al., 2003). Targeted sequences SCY03 and SCP44M51 were not determined in any of the examined rye samples. SCP 16M58 fragment was detected in 72% of the examined lines. SCP16M58 marker sequence was found in six examined SF-lines, therefore, these SF-lines can be used as fertility restorers for MS-forms. Required genomes for rye hybrid selection were identified using CMS-forms and MS-lines. F1 hybrids, based on targeted G-type MS-forms and SF-lines L469/3, L469/8, L476/8 with SCP16M58 sequence, linked with fertility restoration genes (Rf), exceeded other hybrids the weight of grain per ear on the average 1.6. As the result of F1 hybrid assays of winter rye, 4 promising combinations were selected and passed to the “Scientific and Practical Center for Arable Farming” for including in the breeding process. The use of targeted MS- and SF-lines reduces greatly obtaining heterotic hybrids, exceeding the standard variety in grain productivity. The research was supported by the Belarusian Republican Foundation for Fundamental Research (Agreement: B13-072).

# **Outlier detection - from ANOVA to REML: An application in rye hybrid breeding towards genomic selection**

poster s3-06

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Many plant breeding companies use ANOVA-based software for routine analysis of field trials. These programs may offer specific in-built options for residual analysis that are lacking in current REML software. With the advance of molecular technologies, there is a need to switch to REML-based approaches but without losing the good features of outlier detection methods that have proven useful in the past. Our aims were to compare ANOVA and REML approaches, to scrutinize the outlier detection method of the ANOVA-based package PlabStat and to evaluate and propose alternative procedures for outlier detection. We provide a detailed explanation how the PlabStat outlier detection methodology can be translated to REML-based software together with the evaluation of alternative methods to identify outliers. The method combining the Bonferroni-Holm test to judge each residual and the residual standardization strategy implemented in PlabStat exhibited good properties to detect outliers in small and large datasets and under a genomic prediction application. We recommend the use of outlier detection methods as a decision support in the routine data analyses of plant breeding experiments.

# **Emerging molecular tools**

(Session 4)

# Rye DArT arrays effectively anchor BACs onto genetic map

poster s4-01

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A physical map is essential for de novo sequencing and provides the necessary context for exploring genome structure and organization. A key point with respect to physical map construction and exploitation is anchoring of BAC clones to high-resolution genetic maps - integration of physical and genetic maps. Many of the currently established anchoring techniques tend to be low-throughput or involve many processing steps. One of proposed methods to speed up addressing of genetic markers to individual BAC clones involves use of microarrays. In this study we applied Diversity Array Technology, the 11520-clone rye DArT-genotyping array and a three dimensional pooling strategy to anchor clones from the BAC library of the rye inbred line L318 on the integrated genetic map of the rye genome comprising over 4000 DArT loci. In total ca. 43 thousand BAC clones were screened (ca. 65% of the rye genome). Following data deconvolution almost 4000 DArT markers (among them 1428 genetically mapped) were addressed to over 2000 BAC clones. Over 700 DArTs were addressed to more than one BAC clone and more than one DArT was addressed to almost 1200 BAC clones. In total ca. 750 BAC clones were anchored on rye genetic map, from 74 for 7R to 144 to for 6R. The available information on DArT markers' genetic map positions, membership in sequencing bins (established in redundancy analyses of rye DArT marker sequences), and also sequences of several BAC clones were used to verify the reliability of DArT marker-to-BAC clone addressing. Obtained results indicate that Diversity Array Technology enables high throughput and reliable rye BAC clone anchoring on genetic map.

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# DArT markers sequences facilitate functional, comparative and structural genomics of rye

poster s4-02

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Diversity Arrays Technology is a reproducible and cost-effective technique, which allows highly parallel genotyping without previous sequence information. For rye a 11520-clone DArT genotyping array was created and successfully applied, among others, for high density genetic map construction and genome-wide germplasm characterization. We analyzed sequences of 6202 (53.8%) rye DArT markers using various bioinformatic approaches. Redundancy analysis assigned a total of 3964 DArT sequences to 1117 bins, 2847 sequences were unique (redundancy ca. 36%). Subsequently, consensus sequences were generated for each bin. Following functional annotation analysis we were able to attribute putative gene functions for more than 1100 unique or consensus DArT marker sequences (ca. 30%). We found also that ca. 500 individual DArT marker sequences exhibited similarity to various regions of *B. distachyon* genome, with ca. 85% of DArTs mapping within genes. According to expectations based on phylogenetic relationships between Poaceae, sequence homology to *O. sativa* genome was found for a smaller number of DArTs (less than 400), almost 51% of them were placed in genic regions. Finally, 1251 DArT marker sequences were incorporated into publicly available genome zippers of rye chromosomes, at 1092 positions in total, linking thus the available rye genomics resources. Obtained results extend the knowledge on rye genome organization and evolution and will facilitate further genomic research in this species.

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# Approaching the Gibberellin Sensitive Dwarfing Gene *Ddw1* in Winter Rye (*Secale cereale* L.)

poster s4-03

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Winter rye (*Secale cereale* L.) is an economically relatively small yet ecologically valuable agricultural crop, which enables the reliable production of feed, food and agricultural biomass even on light, drought prone soils. The improvement of lodging tolerance counts among the major breeding goals in rye. The main genetic approach to overcome lodging is a reduction of plant height by exploiting dwarfing mutants. The potential of the gibberellin-sensitive, dominant dwarfing gene *Ddw1* for breeding highly productive hybrid rye varieties has yet not been elucidated, as an efficient and reliable method to distinguish homozygous and heterozygous dwarf genotypes is yet not available. Furthermore, an association of *Ddw1* with undesired genes has been reported. The application of molecular markers provides information, which enables to break up complexes between desired and undesired gene variants. We aim to optimize the resolution of marker assisted selection for *Ddw1*. A high-resolution mapping population was used for validating the mode of inheritance of *Ddw1*. A synteny-based mapping-by-sequencing approach on genomic DNA (DArTseq) and cDNA (massive analysis of cDNA ends, MACE) from contrasting bulks of wild-type and mutant genotypes allowed to identify SNPs, which are currently used to establish novel *Ddw1* markers. These markers will be used to construct a high-resolution genetic and physical map of *Ddw1*, which will be used to identify the functional nucleotide polymorphism within a candidate gene that determines the *Ddw1* phenotype. General outcome of this research will be efficient means for identifying sequence variation in genes that control agronomic traits in the complex rye genome. Knowledge achieved in this project will help to exploit allelic variants from germplasm accessions, which can be used for the genetic improvement of adapted gene pools in rye.

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# Identification and genetic mapping of the gene associated with dwarfism of rye plants

poster s4-04

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Rye (*Secale cereale* ssp. *cereale* L.) is a bread crop very important for East and Central Europe as well as Scandinavian countries. In Poland, it takes the third position as regards the sowing area, following wheat and triticale. One of the important problems in rye cultivation is still too big a plant height (often about 140cm). It is often the cause of plant lodging, especially in hard weather conditions such as strong wind or rain. As a result, it suffers the loss of yield quality and quantity, due to preharvest sprouting and the impact of diseases. Susceptibility to lodging depends on straw anatomy and root construction.

One of methods leading to shorter plant straw is the introduction of dwarfing genes to cultivars. This method was successfully used in wheat and triticale, resulting in many semi-dwarf cultivars. In triticale the lodging problem was solved mainly with the introduction of rye *Dw1* gene. Unfortunately, this gene could not be used in rye breeding due to its very negative influence on yield components. So, in rye there have been no cultivars with introduced dwarfing genes until now. Hence, the search for new effective dwarfing genes seems to be very important for this species. In rye only three dominant (*Dw1*, *Dw2* and *Dw3*) and a number of recessives dwarfing genes have been identified. Most of them have been localized in chromosomes arms, but not accurately, and in most cases there are no highly linked markers.

The aim of the study was identification of the new recessive dwarfing gene of rye (named here as *dw9*) and its genetic mapping with DArT markers.

The material of the study were: 1) rye inbred lines: maternal 541 (the normal height) and paternal BK-1 (the dwarf line) obtained from Bashirskaja karlikovaja (VIR, St. Petersburg) and 2) 92 plants of F<sub>2</sub> mapping population obtained from crossing 541×BK-1. The height of all plants was measured. The results obtained from DArTseq analyses were used to construct a skeletal genetic map in JoinMap 3.0 and Multipoint 3.2 programs. From all obtained 42454 SilicoDArTs and 10979 SNPs, only 3493 and 1977 were used for mapping, respectively. Using data from DArTseq mapping of another mapping population (Stojałowski, unpubl.), it was established that the *dw9* gene is located on the 6RL chromosome. The constructed genetic map included a fragment of 6RLas well as 148 SNPs and SilicoDArTs. 17 markers were mapped at the distance to 10cM, 14 of which were SNPs and only 3 – silicoDArTs. The strongly linked SNP marker was linked at the distance of 0,7cM with the *dw9* gene. Thereafter, markers linked with *dw9* gene on the distance to 5cM (7 markers) will be converted into SCAR markers and their segregations will be added to the genetic map constructed in this study.

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# Transcriptional gene silencing approach as a tool to dissect biological function of *ScBx1* in rye

poster s4-05

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Benzoxazinoids (BXs) are defensive compounds of metabolism found in several species of *Poaceae*, including the major agricultural crops: maize, wheat and rye. They were also found in wild barley species *Hordeum lechleri*. The compounds were found to be involved in plant allelopathic interactions and stress tolerance. The most important BXs synthesized in rye is DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one). The compound is the final product of benzoxazinoid biosynthetic pathway catalyzed by five enzymes encoded by *Bx1*–*Bx5* genes. The goal of the project was to experimentally verify the biological activity and function of isolated rye gene. The gene designated as a putative *ScBx1* has high homology to wheat *TaBx1*. The amino acid sequence encoded by the putative *ScBx1* is highly similar to indole-3-glycerol phosphate lyase – the enzyme of the first step of DIBOA biosynthesis pathway. The virus-induced gene silencing (VIGS) system was selected as an experimental tool for functional analysis of this gene. Selected fragments of promoter regions were cloned into cDNA of modified  $\beta$  and  $\gamma$  subunits of *Barley stripe mosaic virus* (BSMV). The resultant plasmids BSMV: $\alpha$ , BSMV: $\beta$ \_fragm.1 and BSMV: $\gamma$ \_fragm.2 were used as the templates for in vitro transcription. The mixture of BSMV: $\alpha$ , BSMV: $\beta$ \_fragm.1 and BSMV: $\gamma$ \_fragm.2 transcripts were used for inoculation of rye seedlings cultivar Konto F1. The mixture of BSMV: $\alpha$ , BSMV: $\beta$  and BSMV: $\gamma$  transcripts (with no cloned fragments) were used for inoculation of rye seedlings cv. Konto F1 and these plants served as the control for all analysis. Leaves with symptoms of BSMV infection were collected 14, 21 and 99 days post inoculation (dpi) and used for: 1) expression analysis of *ScBx1*, 2) detection of CG methylation in the promoter regions selected for cloning and 3) analysis of total amount of DIBOA. Relative expression of *ScBx1* in experimental plants was strongly depressed compared with the control. In leaves collected 14 dpi the values ranged from 0.03 to 0.09, 21 dpi the values ranged from 0.14 to 1.33 and 99 dpi from 0.02 to 0.67. *ScBx1* promoter methylation in the experimental plants was significantly enhanced compared with the control plants. Enhanced promoter methylation correlated with lowered expression of *ScBx1*. The methylation rate of the target region 14 dpi ranged from 1.3% to 2.0%, 21 dpi ranged from 3.1% to 34.9% and 99 dpi from 12.2% to 33.9%. The average methylation in control plants was 1.6% and it was similar in leaves collected 14, 21 and 99 dpi. The amount of BXs in plants with lowered *ScBx1* expression was significantly lower compared with controls. The amount of BXs in experimental plants (i.e. VIGS inoculated, with enhanced *ScBx1* promoter methylation and lowered *ScBx1* expression) in leaves collected

14 dpi ranged from 756.5 to 993.1, 21 dpi ranged from 172.9 to 635.5 and 99 dpi ranged from 80.5 to 216.1  $\mu\text{g/g}$  of d.w. BX concentration in control plants in leaves collected 14 dpi ranged from 2787.1 to 1136.6, 21 dpi ranged from 2539.3 to 846.7 and 99 dpi ranged from 1003.1 to 326.4  $\mu\text{g/g}$  of d.w. The results indicate that VIGS-BSMV system can be used as an efficient tool for functional analysis by means of siRNA directed CG methylation of genomic DNA and transcriptional silencing of a selected gene. The correlation between low ScBx1 expression and low BXs content observed in experimental plants confirms that the analyzed gene encodes indole-3-glycerol phosphate lyase, which is active in DIBOA synthesis pathway in rye.

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# Identification and chromosomal location of new, dominant dwarfing gene in rye

poster s4-06

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The research material was generation F2 consisted of 500 plants, created in the result of crossbreeding between inbred lines: 541 (S30) of the typical height (140 cm) and a dwarf line S44 (S2) (approx. 85 cm), originated from population S44 (Accession 31147, The Plant Breeding and Acclimatization Institute (IHAR) - National Research Institute Radzików). Additionally a field experiment has been created which allowed to compare the phenotypic effects of dwarfism gene (generation F1 of inbred line S44 and cultivar Dańkowskie Amber) relative to the standard (Dańkowskie Amber).

All plants of F2 generation were first evaluated visually and measured. The observed segregation was consistent with monogenic inheritance model. Random sample of 92 plants and parental forms were genotyped using DArT-Seq technology. Based on the information about the location of DArT-Seq markers (Stojałowski, unpublished) genetic maps of all seven chromosomes of rye have been created. The segregation of dwarfism in mapping population has been used for gene location. The gene was mapped on the long arm of chromosome 3R, near the centromere. Since this is a new, so far not cataloged gene, it was given a new number (*Dw4*).

Also a preliminary assessment of the impact of this gene on morphological traits has been made by analyzing the rye hybrid plants F1 (S44 × Dańkowskie Amber). Measurements of the following traits have been made: plant height, length and thickness of the all internodes, spike length, number and weight of seeds per spike and TGW. The average height of hybrids does not exceed 80 cm and was lower than the standard by approx. 44%. It was found that all hybrid internodes were proportionally shorter than the internodes of Dańkowskie Amber variety. There was no negative effect of the *Dw4* gene on the length of the spike and the number of seeds per spike. At the same time a reduction in seed weight per spike (approx. 10%) and TGW (approx. 20%) has been observed.

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# **Relationship of GA3ox and GAMYB with spike elongation in rye (*Secale cereale* L.)**

poster s4-07

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Divergent selection carried out among 300 RILs representing F6 generation of the cross between 541 and Ot1-3 lines allowed to distinguish two subgroups of RILs with long (10.04-13.40 cm) and short (6.48-8.52 cm) spike. Bidirectional Selective Genotyping (BSG) of the GA3ox and GAMYB polymorphic loci showed distorted segregation of both genes within 20 RILS with short spike. This result suggest that GA3ox and GAMYB genes are involved in spike elongation process. Surprisingly, alleles of parental line with long spike (541) were associated with shortening of spike length demonstrated by 20 selected RILs. This observation suggest that both loci are regulated by similar mechanism affecting GA production.

# Genetic mapping of recessive dwarfing gene *dw8* in rye

poster s4-08

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Lodging in cereals leads to a significant decrease in both quality and quantity of grain yield, generating serious financial losses. Lodging can usually be triggered by adverse weather conditions such as heavy rains, strong wind or by excessive nitrogen fertilization. Improvement of resistance to lodging is one of the major goals of cereals breeding. Lodging resistance in rye is highly correlated with its morphological traits such as: length of the blade, length and width of internodes and structure of the root system. Height reduction in cereals can easily be achieved by treatment with growth retardants. Another way of height reduction is introduction of dwarfing genes into a plant of a normal height by crossing it with a short straw plant. There are three dominant dwarfing genes in rye (*Dw1*, *Dw2* and *Dw3*) and a number of recessive ones. Most of them were localized on chromosomes but their exact positions remain unknown. There are four dwarfing genes identified on chromosome 5R: dominant gene *Dw1* (EM-1 mutant) and recessive genes: *dw6* (I-G-type), *ct2* (Moskowskij Karlik ) and *dw8* (found in RXL10 inbred line). The aims of this study were: precise localization of recessive dwarfing gene *dw8*, evaluation of its influence on plants height and yield-forming characteristics as well as assessment of sensitivity to exogenously applied gibberellin.

Plant material consisted of rye inbred lines: (I) 541 (normal height) and RXL10 (dwarf phenotype), (II) F2 mapping population derived from crossing 541 × RXL10, (III) extreme groups consisting of twenty three high and twenty three short straw F4 recombinant inbred lines (RILs), (IV) F1 and F2 hybrids derived from crosses of Dańkowskie Amber × RXL10. Several marker types were used for mapping: SCAR, SSR, DArT-SCAR, SNP, EST-SNP and DArTseq. Their correlation with height characteristic was assessed by BSG method, and confirmed by  $\chi^2$  test. On the basis of the obtained results genetic maps of 5RL chromosome for F2 and F4 populations were created. Biometric measurements were conducted on Dańkowskie Amber and Dańkowskie Amber × RXL10 hybrids of F1 and F2 generations. Dwarf phenotype plants of F2 segregating population were on average 49.1% shorter in comparison to Dańkowskie Amber. Gibberellic test showed that RXL10 inbred line carrying *dw8* dwarfing gene is sensitive to exogenously applied gibberellin.

*This work was supported by the National Centre for Research and Development under a grant PBS1/B8/5/2012.*

# **The search for molecular markers linked to the dominant gene *Dw1* for dwarfness in rye (*Secale cereale* L.)**

poster s4-09

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Dominant dwarfing genes in rye have been previously identified and located on chromosomes 1RL (*Dw3*), 5RL (*Dw1*) and 7R (*Dw2*). However, only *Dw1* gene was found to be of practical importance in rye, as in triticale cultivation. *Dw1* was identified and selected from the Russian population of rye EM1. The aim of the study was to search for new molecular markers closely linked to *Dw1* locus, and to assess their suitability for efficient selection of dwarf genotypes. The research material consisted of 500 individuals of the F2 population obtained from propagation of offspring of inbred lines: 541 (S30) with normal height (140 cm) and dwarf line EM1 (S2) (approx. 85 cm). Segregation of trait 'plant height' observed within the F2 progeny differed from the monogenic inheritance model. Genotyping with the use of DArT-Seq technology was carried out for a set of genotypes including high plant, dwarf plant and parental lines. Genetic maps of all seven chromosomes of rye were constructed on the basis of information about chromosomal location of selected DArT-Seq markers (Stojałowski, unpublished). Analysis of co-segregations of *Dw1* dwarfism gene and selected/putative molecular markers enabled identification of four DArT-Seq markers closely linked to *Dw1* locus. Among them, one, converted to the co-dominant marker, was mapped on 5RS 0.9 cM from *Dw1* locus. In the next stages of the project, critical verification of usefulness of dwarf rye plants for selection with the use of identified molecular markers will be done.

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# Morphological and molecular characteristic of rye NILs differing in recessive dwarf gene

poster s4-10

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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 S4 generation which was due to the presence of the recessive allele. The pair of sublines (typical and dwarf variant) were selected from the segregating heterozygous high plants of S5 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. Three molecular techniques (DArT, DArTseq and RAPD) were used to assess genetic diversity of NILs and to find markers for dwarf gene. 3739 DArTs were obtained and 3 of them were polymorphic. There were 59672 SilicoDArT (DArTseq) markers obtained and 618 of them were polymorphic. Among 19049 SNP (DArTseq) markers 7 were polymorphic in investigated sublines. Out of 1 445 RAPDs tested, none were polymorphic for selected NILS. The results indicate a very high genetic similarity of obtained NILs. DArT-seq technology and F2 mapping population, derived from hybrid between typical and dwarf NIL were used to establish a position of analysed gene in the rye genome.

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# Assessment of genetic diversity of Polish rye (*Secale cereale* L.) breeding materials with the use of DArTseq genotyping system

poster s4-11

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In hybrid breeding, the heterosis effect significantly depends on genetic diversity of parental components. The substantial progress achieved in rye hybrid breeding in Germany has proved that parental components should be developed from genetically divergent gene pools, to make maximum use of heterosis (Geiger and Mediner, 1999).

Molecular techniques enable precise measurement of the genetic diversity between different accessions or groups of germplasm. The technology of DArTseq utilizes DArT marker platform in a combination with next-generation sequencing platforms and represents a new implementation of sequencing of complexity-reduced representations and more recent applications of this concept on the next-generation sequencing platforms. So far, DArTseq has been successfully applied for genetic diversity assessment studies in several plant species.

The aim of this study was to assess the genetic diversity of Polish rye breeding materials using DArTseq genotyping system. The material consisted of 150 inbred lines derived from several scientific institutions (Department of Plant Genetics, Breeding and Biotechnology Warsaw University of Life Sciences in Warsaw, Department of Plant Genetics, Breeding and Biotechnology West Pomeranian University of Technology in Szczecin, Department of Genetics, Plant Breeding and Seed Production Wrocław University of Environmental and Life Sciences), and breeding companies (Danko Plant Breeders Ltd. and Poznań Plant Breeders Ltd.). The analyzes comprised a total of 92209 silico DArT and 36894 SNP markers. Based on genotyping results, the genetic similarity coefficients according to Jaccard were calculated followed by hierarchical clustering and determination the population structure.

Our study showed no correlation between clustering and inbred line origin except for Breeding materials derived from Wrocław University of Environmental and Life Sciences which constituted a clearly distinct group. Inbred lines from Poznań Plant Breeders Ltd. were the group characterized by the highest genetic diversity.

1. Geiger HH, Miedaner T (1999) Hybrid rye and Heterosis. In: Coors, JG and S Pandey (eds) Genetics and Exploitation of Heterosis in Crops, Crop Sci Soc America, Madison, Wisconsin, USA, pp 439-450.

# **Use of genetic resources and cytogenetics**

(Session 5)

# **Introgression of genetic material of wheat (*T. aestivum* L.) into genome of tetraploid rye (*S. cereal* L.)**

poster s5-01

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The prospects of using interspecific introgressions in cereals breeding are verified by the results obtained in wheat: 650 commercial varieties, containing wheat-rye translocations in the genome are known in the world gene pool of wheat (*T. aestivum* L.). No introgressions of wheat genetic material were revealed in the rye genome.

We have developed a methodical model of wheat genetic material introgression into the rye genome based on tetraploid rye (RRRR,  $4x=28$ ) hybridization with an intermediate species and the wheat genome source – hexaploid triticale (AABBRR,  $6x=42$ ), followed by backcrossing of the hybrids F1 (RRABR,  $5x=35$ ) with self-fertile (sf) tetraploid rye. It is based on the genetic factors: (1) specific genome and chromosome composition of the rye-triticale hybrids F1 (RRABR,  $5x=35$ ); (2) the promoter effect of a triple dose of the rye genome on homoeologous chromosome pairing in meiosis in the hybrids F1 and the possibility of wheat genetic material introgression into the rye genome; (3) formation of viable gametes with a different chromosome composition in the hybrids F1 (major diploid rye genome (RR) – a factor of partial meiosis normalization).

The rye-triticale hybrids F1BC1 with the chromosome number from 28 to 38 were obtained. The expected recombinant forms of tetraploid rye were selected in F3-F5BC1 and availability of wheat genetic material in them was analysed using a universal PCR-based marker for the wheat genome. The research results of wheat chromatin introgression into the tetraploid rye genome are discussed.

# **Progress in the breeding of rye hybrids for fertility restoring ability in the Pampa cytoplasm**

poster s5-02

I..Kolasińska

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The objective of this study was to determine frequency of fertility restorers among rye genotypes developed from newly created breeding populations. Restoring ability of numerous S2 and S3 inbred lines and synthetic populations were studied in the years 2013 and 2014. The inbred lines were crossed to a hard-restoration tester (CMS-Tt) under isolation bags. The synthetic populations were crossed to CMS-Tt in plastic tunnels and between plastic walls. The F1 hybrids were growing in plastic tunnels and/or in field conditions. The level of male fertility of test hybrids was assessed by anther dehiscence visual scores of single plants according to the scale of Geiger and Morgenstern (1975). The restoration indices (RI) were calculated according to the formula:  $RI = \% \text{ of male fertile (\%mf) plants} + 1/2 \% \text{ of partially male fertile plants (\%pmf)}$ .

It was found, that majority of inbred lines and synthetic populations were very effective in restoration of male fertility of their test hybrids. Frequency of full restorers among S2 inbred lines assessed in 2013 and 2014 was 36,2% and 46,8%, respectively. Frequency of full restorers (RI=100%) among S3 inbred lines assessed in 2013 and 2014 was 30% and 40%, respectively. Several genotypes were able to restore completely pollen fertility of their test hybrids cultivated in both conditions plastic tunnels and field (RI=100%; %mf=100). Most of synthetic populations proved to be very effective restorers. Frequency of population with restoration indices above 70% among all tested was 60% and 100% in 2013 and 2014, respectively. Utilization of these very effective restorers in breeding programe should improve level of male fertility of newly created rye hybrids.

Key words: hybrid breeding, male sterility, Pampa cytoplasm, restoration of male fertility, rye.

# **New source of fertility restoring genes for the Pampa cytoplasm in winter rye**

poster s5-03

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The objective of this study was to identify effective fertility restorers for the Pampa cytoplasm among rye genotypes originated from Turkish landrace populations and to determine the inheritance of fertility restoration in this source. Numerous BC2S3 inbred lines were developed from populations derived by crossing modern inbred lines (the Smolice breeding program) with Turkish landrace populations. Single plants of inbred lines were crossed to a hard-restoration tester (CMS-Tt) under isolation bags. The level of male fertility of test hybrids was assessed in plastic tunnels and/or in field by anther dehiscence visual scores of single plants with a 1–9 scale. The restoration indices (RI) were calculated according to the formula:  $RI = \% \text{ of male fertile (\%mf) plants} + 1/2 \% \text{ of partially male fertile plants (\%pmf)}$  (Geiger and Morgenstern, 1975). Two full restorer lines (LTR 2 and LTR 28) with good performance per se were crossed to the male sterile line. Completely male fertile F1 plants were self-pollinated by bagging with paper bags to produce F2 seed. Male sterility/fertility of F2 plants was determined as described above. Goodness of fit of observed male fertile to male sterile ratios to an expected segregation ratio for fertility restoration genes was tested by the standard Chi-squared method.

Majority of inbred lines (LTR) derived from these populations proved to be very effective restorers. Frequency of effective restorers among genotypes assessed in 2009 and 2010 was 58,3% and 82,3%, respectively. Several genotypes were able to restore completely pollen fertility of their test hybrids cultivated in both conditions plastic tunnels and field (IR=100%; %mf=100). Segregation ratios of pollen fertility in the F2 populations revealed that a single dominant restorer gene appears to be responsible for fertility restoration in LTR 28. However, segregation ratios in the F2 populations with LTR 2 suggested more complicate inheritance of fertility restoration. Segregation analyses in BC1 and/or F3 progenies could helped in establishing mode of inheritance in this restorer gene source. Both lines LTR 28 and LTR 2 provided high and environmentally stable level of restoration and could be utilized for improvement of male fertility in Pampa-based rye hybrids.

Key words: hybrid breeding, male sterility, Pampa cytoplasm, restoration of male fertility, rye.

# **A shotgun assembly of the long arm of rye chromosome 5R**

poster s5-04

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A. G. Sharpe

Low-temperature tolerance is an important agronomic trait for both spring and winter cereal crops. Winter habit rye displays the greatest low-temperature tolerance amongst temperate cereal crops. Despite the identification of regions conferring low-temperature tolerance in rye and wheat, efforts to exploit the superior low-temperature tolerance of rye for improvement of wheat have been unsuccessful. The cereal group 5 chromosomes, and in particular a region designated as Fr2 have been implicated in low-temperature tolerance. Using a subtractive approach we have assembled a chromosomal translocation of the long arm of rye variety "Puma" chromosome 5R along with chromosome 5A of the wheat variety "Norstar" and used these assemblies for comparative analyses of the Fr2 loci.

# Inheritance of chosen types of dwarfism in winter rye (*Secale cereale* L.)

poster s5-05

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The advantage of dwarf type of crop growth is the increased resistance to lodging. In crops a number of genes determining this feature have been identified. In practical growth of short-stalk varieties, mainly the dominant genes, e.g. in wheat *Rht* genes, are used. For rye, the dominant genes (*Ddw1*, *Ddw2* and *Ddw3*) as well as recessive ones have been identified, but none of them has been applied in the growth of dwarf varieties of rye (Kobyljanski 1972, 1975a, Stojalowski et al. 2005). However, the gene *Ddw1* identified in rye has been successfully used for the growth of semi-dwarf varieties of triticale (Wolski & Gryka 1996). In this paper, the dwarf inbred lines of rye were characterised and the pathway of their inheritance was described. Two of them were obtained as a result of inbred growth of cultivated varieties (mk i jeź), while one was a recombinant in the segregating generation (kn). These lines had reduced stem length, one of them showed a not compact type of growth, which prevents dense population of plants over a given area.

On the basis of observation of the offspring of F2 generations and backcrossing it was shown that this feature in the inbred rye lines studied was determined by the recessive genes, denoted as *ds3*, *ds4* and so.

A subsequent stage of the study was the use of PCR with the starters SSR and ISSR for evaluation of the lines polymorphism and offspring of the segregating generations. The polymorphism detected with the use of ISSR markers in the inbred lines of rye was found to be greater than that detected with the microsatellite markers SSR. The SSR and ISSR markers differentiating the segregating generations were proposed. One of this type of dwarfism can be used in breeding.

# Lithuanian rye breeding and results

poster s5-06

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In Lithuania, the breeding of winter rye, like that of other cereals, was started by prof. Dionizas Rudzinskas in 1922. During the first 10-year period, winter rye breeding was carried out on a small scale. From 1932 onward, local rye varieties or populations were collected from different localities of Lithuania and were investigated at the Dotnuva Plant Breeding Station. Unfortunately, the collected material proved to be of low value.

During the post-war period, the basic method used in winter breeding in Dotnuva was intervarietal hybridization combined with individual selection within a family. As a result the following winter rye varieties have been developed and registered:

**Dotnuvos Aukštieji.** Selected from a local variety 'Rygiskieji'. It was included in the National Variety List from 1950 to 1964. The breeder is D.Rudzinskas.

**Lietuvos 3.** (Viatka/Dotnuos VIII). This variety was registered in Lithuania from 1957 to 1977. In 1962 it was included in the Variety Lists of Belarus and Ukraine. The breeder is P.Virbickas.

**Baltija.** (Sangaste/ Dotnuvos VII). The variety was registered from 1962 to 1976. The breeder is P.Vinickas.

**Kombaininiai** (Viatka/Petkus short). In 1973 it was registered in Lithuania and in 1974 – in Belarus. The breeder is P.Virbickas.

**Rūkai DS.** (4n) (Em-1/Dotnuvele//Dotnuvele) has been registered in Lithuania since 2011. The breeders are V.Plycevaitiene, V.Ruzgas.

**Duoniai.** (Em-1/Saratovskaja//Voschod/Kustro. The variety was registered in Lithuania from 1994 to 2007. The breeders are V.Plycevaitiene, J.Sprainaitiene.

**Joniai.** The variety was developed from a cross between Borba and Duoniai. It has been registered in Lithuania since 2002. The breeders are V.Plycevaitiene, V.Ruzgas.

**Virgiai.** The variety was developed from a cross between Amilo and Motto. It has been registered in Lithuania since 2010. The breeders are V.Plycevaitiene, V.Ruzgas.

The area cultivated with rye in Lithuania has been decreasing since 1993. Seed companies prefer hybrid rye varieties and the area sown with population varieties is steadily diminishing.

# Seed Production of Rye in Lower Silesia Voivodeship – Poland

poster s5-07

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In Poland the cultivation of rye has generally extensive character, which is characterized by her relatively low expenditure of means of production per unit area. In addition, this species has a relatively small demand of soil, water and is marked by low sensitivity to previous crop. These features causes that rye still takes considerable acreage, although observable downward trend for several years. The winter rye crop dominates in cultivation, while spring is the form of marginal importance.

The aim of the work was to present, over the years, the situation of seed production of *Secale cereale* in climatic-soil conditions of Lower Silesia - the northwestern part of Poland. According to database available by the State Plant Health and Seed Inspection Service in Wrocław, the seed production area and the average yield of rye in Lower Silesia Voivodeship between years 2009-2012 was following: 2451,87ha and 63,81q/ha (2009); 1149,31ha and 69,35q/ha (2010); 988,38ha and 60,30q/ha (2011); 1701,32ha and 61,45q/ha (2012). The area of winter rye seed crop stands in Lower Silesia Voivodeship for several years is moderately stabilized and includes in the area of about 1,500 hectares, with an average yield of 63 quintals per hectare. The spring rye, in contrast, has a marginal meaning. In seed production of rye in this region dominates the following seed companies: KWS Lochow sp. zo. o. in Kondratowice and SAATBAU POLSKA sp. zo. o. in Środa Śląska.

In Lower Silesia Voivodeship dominates the production of winter wheat. However, it should be noted, that the cultivation of rye is increasingly important, and the area of rye cultivation is gradually increasing by Lower Silesian farms. Consequently, seed companies have to meet the demands for seed material of the expected parameters. The results show the tendency connected with cultivation of seed material in a defined part of Poland. According to database, since the year 2010 the area cultivation of seed material of cereals has been gradually increasing in Lower Silesia Region, but the average yield per hectare is stabilized. There is clearly a great need to produce certified seed material. There has been a noticeable growing awareness of farmers about the benefits and profits resulting from the use of high quality seeds produced by seed companies.

The seed companies and farms producing seed material in Lower Silesia Voivodeship show great care and diligence and also use the newest technology in production of the highest seed quality.

# **Disease resistance and tolerance to abiotic stresses**

(Session 6)

# **Structural and expressional analysis of *ScBx3* and *ScBx4* genes involved in benzoxazinoid biosynthesis in rye (*Secale cereale* L.)**

poster s6-01

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Benzoxazinoids (BXs) are protective and allelopathic secondary metabolites found in numerous species, predominantly belonging to the *Poaceae* family. They also have many other unique properties including anti-bacterial and anti-fungal activity, and the ability to reduce alfa - amylase activity. We isolated and sequenced two genes: *ScBx3* (acc.No. KF636827) and *ScBx4* (acc.No. KF636826) which encode monooxygenases controlling the biosynthesis of 3-hydroxyindolin-2-one and 2-hydroxy-1,4-benzoxazin-3-one, respectively. The lengths of genes *ScBx3* and *ScBx4* including exons, introns and 3'UTRs were 1863 and 2030 bp, correspondingly. Each of the genes comprised three exons and two introns and they were arranged "tail-to-head", and the distance from the last nucleotide of the 3'UTR of *ScBx4* to the first nucleotide of the first exon of *ScBx3* was 8073 bp. The same arrangement was observed by Nomura et al. (2008) for *TaBx3* and *TaBx4* genes in all three genomes of hexaploid wheat. We also assessed the expression of *ScBx3* and *ScBx4* in two weeks old seedlings of a set of diverse lines of rye (DIL), which represent the variability of Polish breeding materials. Specific primers targeting actin (reference gene) and genes of interest were chosen for the amplification. Those specific primer pairs were successfully employed in real-time PCR conjugated with SYBR® Green I dye for the direct quantification.

The lengths of the amplicons varied between 100 and 105 base pairs.

The expression patterns and relative transcription levels of *ScBx3* and *ScBx4* varied considerably among genes as well as DILs analysed.

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# Assessment of the rye breeding material in respect of immunity to infections caused by the powdery mildew *Blumeria graminis*

poster s6-02

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The powdery mildew *Blumeria graminis* DC invading crops represents one of the most devastating and commonly occurring diseases of cultivated cereal plants. There exists a negative correlation between affection of plants by this pathogen and their yielding. It is particularly well manifested in varieties and lines characterized by a shortened leaf for the fact that in the rye – accompanying the leaf shortening – the proportion of leaves in the overall balance of assimilation increases, and since the infected leaves do not participate in the process, this leads to decline in the productivity of a plant. The powdery mildew produces forms that are specialized in invading particular plant species. The eight specific forms distinguished in this pathogen species include *Blumeria graminis* f. sp. *secalis*, which attacks the rye. Research material, made up by 125 winter rye genotypes obtained from breeders (Choryń, Laski, Sobiejuchy), comprised inbred lines, populations and standard varieties Agrikolo and Bosmo. The estimate of the susceptibility of rye genotypes to infection by *Blumeria graminis* was performed based on a glasshouse experiment with unnatural inoculation. A four-degree scale of infection (0–3) was employed, where 0 – denoted lack of symptoms, whereas 3 – very extensive infection, with colonies of the powdery mildew occupying 75–100% of the leaf blade surface. The level of the plants affection was determined two weeks after inoculation. The performed analysis of variance has shown significant diversification of the studied rye genotypes with respect to their resistance to infection by the powdery mildew, and a comparison of objective means with the Duncan test has allowed to divide the rye genotypes into five overlapping uniform groups. The tested genotypes, sent for the study by breeders, included objects characterized by high resistance (CHD Ma 222, CHD Ma 223, CHD Ma 224, CHD Ma 227, CHD Ma 233, CHD Ma 239, CHD Ma 240, SOA Mącz 117 oraz SOA Mącz 146). These genotypes can constitute good initial material for breeding cultivars that would be immune to the pathogen concerned. Selected resistant genotypes and the most vulnerable ones were tested for the presence of markers linked with the genes of immunity to the powdery mildew. As a result of the PCR reactions carried out with the use of starters proper to the marker specific to gene *Pm3f*, products of desired size were acquired both for the lines resistant and ones susceptible to infection by *Blumeria graminis*. However, in the two groups additional products of amplification were detected. Production of nonspecific amplification products was also recorded for PCR reactions conducted with the employment of starters proper to marker STS638 (*Pm1*), and to markers specific to genes *Pm3b*, *Pm3c*, *Pm3d* and *Pm3g*. An analysis of the obtained amplification products for marker Xcfd81-5D (*Pm2*), and those specific to *Pm3a*, Xgwmc356 (*Pm4a*), ResPm4 (*Pm4*), Xgwm159 (*Pm16*), IAG95 (*Pm17*), Xbarc144 (*Pm34*) and Xwmc41 (*Pm43*) has revealed presence of a product of the predicted size, but the pertinent markers were found both in the lines of the lowest and the highest

degree of infection by the powdery mildew. Of all the examined molecular markers linked with genes of immunity to *Blumeria graminis*, this is exclusively marker ResPm4 that occurs only in the winter rye genotypes resistant to infection by the pathogen concerned. This particular marker can be used in selection of the rye breeding material having the *Pm4* resistance gene. Markers that yield specific amplification products characteristic of the markers linked with resistance genes *Pm2*, *Pm4a*, *Pm16*, *Pm17*, *Pm34* and *Pm43* are worth notice, but they have been detected both in tolerant genotypes as well as those susceptible to affection by the powdery mildew. Detection of the existence of markers linked with the genes mentioned can testify to the presence of these genes in the winter rye analyzed genotypes, yet, they do not guarantee complete immunity to the pathogen's population that was used for artificial inoculation.

# Identification of the genetic traits responsible for 'Stabilstroh' phenotype

poster s6-03

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Lodging, the state of permanent displacement of tillers from their upright position, decreases grain quality and increases the costs of harvesting, qualifying this phenomenon as one of the most serious problems in cereal crop production. Therefore lodging resistance is an important agronomic trait, especially in rye (*Secale cereale* L.), where yield losses due to lodging can be as high as 75%. 'Stabilstroh', a recently identified genotype of rye, not only has the best lodging resistance, but simultaneously it is characterized by the longest tillers among the German cultivars of rye hybrids.

In order to identify the genetic traits responsible for the 'Stabilstroh' phenotype histological and ultrastructural investigations were focused on the most prone to lodging basal internodes of segregating F2 population ('304/1') and its parental lines: 'ms135' ('Stabilstroh') and 'R1124' (wild type). Analyses of tissue distribution, cell size, and cell wall thickness using light microscopy, scanning electron microscopy, and transmission electron microscopy revealed cell walls of sclerenchyma and the inner periclinal cell wall of the epidermis to be thicker, more lignified, and more structured in the 'Stabilstroh' genotype as compared to the wild type. 'Stabilstroh' is also morphologically characterized by pronounced stem invaginations and a significantly higher ratio of sclerenchyma to parenchyma tissues (sc/pa ratio), important factors enhancing mechanical stability of the crop stem. Not only do these features improve mechanical properties of lodging resistant genotype, but they are also responsible for increased biomass production. The quest for QTLs (Quantitative Trait Loci) for improved lodging resistance was based on the inheritance of microsatellite (SSR) and DArT markers, and revealed around 450 markers linked to the traits affecting mechanical stability of tillers.

# Assessment of the rye breeding material resistance to infection by the brown rust *Puccinia recondita* f. sp. *Secalis*

poster s6-04

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The brown rust occurs each year, in a greater or smaller intensity, on all populations and hybrid varieties of the winter rye, and also of the spring rye. The causing factor of this disease is the fungus *Puccinia recondita* f. sp. *secalis*, and the infection is particularly dangerous to the cultivars of shortened leaf, in which the share of leaves in the overall assimilation balance is more considerable than in the high varieties. The harmful effect of the brown rust begins during the sporification phase of this pathogen since at that time the activity of the enzyme dehydrogenase declines whereas the ribonuclease activity significantly rises and the amount of nucleic acids in leaves decreases. The flaming symptoms of the rust that appear on the leaf surface reduce the assimilation area, which, consequently, contributes to a decline in the productivity of plants. The pursued molecular research and attempts at mapping the genes that determine immunity to *Puccinia recondita* f. sp. *secalis* in the rye enabled to develop molecular markers RAPD and SSR linked with gene Pr1, located on chromosome 6RL, and with gene Pr2 on chromosome 7RL, an advantage to be made use of in selection of material for breeding. The study material was composed of 219 winter rye genotypes acquired from breeders, including inbred lines, populations and standard varieties Agrikolo and Bosmo. The estimate of the susceptibility of the rye genotypes to infection by the brown rust was performed by the laboratory method. Leaves of ten-day rye seedlings were placed on a medium, infected with the prior prepared inoculate of the pathogen, and after 10 days the level of affection was assessed with the application of a four-degree scale, where 1 – no affection, 4 – strong symptoms of infection by the brown rust visible on a leaf. An analysis of the intensity of the brown rust infection in leaves under the conditions of unnatural inoculation has revealed that the genotypes studied were characterized by low immunity to the pathogen concerned. There were merely two objects without traces of *Puccinia recondita* f. sp. *secalis* spores on leaves (NS 16/12, NS 17/12), which can be regarded as resistant to infection by the pathogen and eight were recognized as tolerant (CHD Ma 237, CHD Ma 238, CHD Ma 213, WS 14/12, WS 15/12, NS 19/12, NS 20/12, NS 80/12). For molecular analyses, DNA isolated from the genotypes displaying immunity in laboratory tests and from standard varieties Bosmo and Agrikolo was used. The starters employed had been created based on sequences of genes responsible for the immunity to the brown rust in the rye (genes designated as LR) and wheat (genes designated as TC), such as Lr1, Lr9, Lr24, Lr47, Lr10, TC680078, TC72745, TC76051 and TC77841. The PCR reaction with the application of starters developed for genes Lr1, Lr9, Lr24, Lr47 did not yield unequivocal outcome for amplification of the expected product occurred both in the sensitive forms as well as in those resistant to the brown rust infection. In the group of starters linked with resistance genes in the wheat, marker TC72745 linked with the gene of immunity to *Puccinia recondita* f. sp. *secalis* was distinguishable. As a

result of amplification reaction, markers of desirable size were obtained for the genotypes of the highest immunity to infection in laboratory conditions. No marker was detected in the forms susceptible to infection by the brown rust. Due to a small number of genotypes exhibiting full resistance to *Puccinia recondita f. sp. secalis*, the effectiveness of the search for resistance genes with the application of the TC72745 marker and microsatellite markers should be checked on a larger sample of immune forms in order to verify its stability.

# Increasing spread of *Claviceps purpurea* (fr). Tul. and its effect on the quantity and quality of winter rye

poster s6-05

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Problem of increasing infection *Claviceps purpurea* on winter rye has become very important in many regions of Russian Federation. The analysis showed that until 1996 ergot was not economically significant disease. It was found that over the past 15 years have been reported four strong epiphytoses (2003, 2007, 2011, 2012) and only three years (1998, 1999, 2010) there was no disease. Depressive disease was observed in years with strong summer drought.

One reason for disease amplification is the weather conditions favorable for development pathogen. Correlation between ergot susceptibility and average daily temperature 2 ten days of June (beginning of flowering)  $r = -0,689^{**} \pm 0,256$ , relationship between infestation ergot and average temperature June  $r = -0,614^* \pm 0,196$ . An increase in temperature during flowering rye reduces the defeat ergot. Negative correlation was found between the defeat of ergot and relative humidity  $r = 0,691^{**} \pm 0,176$  (3 ten days of June). Consequently, ergot on rye developed in close connection with environmental factors (amount of precipitation, average temperature and relative humidity).

Regression analysis of long-term data showed that an increase in temperature during flowering rye 1 degree Celsius is reduced ergot infected plants by 0.13%. The regression equation between parameters relative humidity - number of infected plants has the form:  $y = 0,0511x - 2,635$ . Therefore, increasing the relative humidity of air at 1% leads to an increase of 0.05% infestation.

Yield losses consist of direct damage (no grain, instead of which is formed sclerotia) and indirect (as a result of a large number of empty spikelets, adjacent to those which form ergot). Increasing the number of sclerotia in the ear reduces the number of grains per spike and grain weight from the ear ( $r = -0,76 \dots -0,80$ ). Significant ergot damage on the rye ear productivity been set. The increase in infestation of ear 1 sclerotia reduced the weight of grain from the ear to 0.19 g and the number of grains per ear by 4.73 (mean of 7 years, 2005-2011).

Prerequisite for the successful breeding of winter rye for resistance to *Claviceps purpurea* is to identify resistant samples from the collection of VIR. During 2005-2008 were estimated more than 90 varieties in natural infectious background. Most of the Russian samples were moderately susceptible varieties. Infestation of grain mass ergot sclerotia varied from 0.01 to 0.76%.

Were identified 5 Russian (IL 23/94, Ilmen, Kama 3, Siberian, 82, Ržanka) and 7 foreign varieties (Pallada, Pudmericke, J03374, P-8, A-8, Frederick, Gaderovo) with the smallest defeat ergot (0,01-0,03%) combined with resistance to another diseases and other valuable parameters. Among of our population of rye for resistance to ergot should be allocated variety Tatar 1. The mechanism of resistance to ergot this variety is a great synchronicity of stems, a short time of flowering and high pollen shedding ability.

# Temperature dynamics influence on the yield of winter rye

poster s6-06

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Within 25 years of an assessment (1990-2014) of exemplars of a winter rye in competitive test of 5 years received productivity about 2 t/hectare, 11 years – at the level of 3-4 t/hectare and 9 years of 5 t/hectare and above. The analysis of correlative dependence for all years revealed low negative influence of average temperature autumn ( $r = -0,32$ ) and summer ( $r = -0,33$ ) stages of vegetation, the sum of positive temperatures in the summer ( $r = -0,34$ ) and the negative in the winter ( $r = -0,30$ ), soil freezing depths ( $r = -0,37$ ) at a size of productivity of a rye. The negative influence of duration of winter ( $r = -0,59$ ) and positive is established to duration of summer vegetation ( $r = -0,39$ ).

The assessment of rye productivity dependence on the average temperature of month of vegetation showed positive influence of temperature increase in November ( $r = 0,331$ ) and February ( $r = 0,434$ ), the negative of hot weather in June ( $r = -0,781$ ).

Influences of a temperature factor in years with the different level of productivity considerably differed. In years with low productivity the negative temperature effect in October ( $r = -0,523$ ), January ( $r = -0,506$ ) and July ( $r = -0,706$ ), positive in December ( $r = 0,471$ ), March and April is established ( $r = 0,824$ ). In years with average productivity temperature effect was expressed more weakly, so low negative impact is established in May ( $r = -0,341$ ) and positive in December ( $r = 0,403$ ), February ( $r = 0,314$ ) and March ( $r = 0,563$ ). In years with high productivity the negative temperature effect in February ( $r = -0,763$ ), positive in months of autumn ( $r = 0,32-0,49$ ) and summer ( $r = 0,427-0,558$ ) vegetation is established.

As a result of researches concluded that in years with low productivity of a winter rye of temperature variation were among factors which created adverse conditions for formation of efficiency of plants.

# Dependence of the yield of winter rye on dynamics of loss of rainfall

poster s6-07

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The average yield of winter rye exemplars in nursery of competitive test from 1990 to 2014 changed from 1,58 t/hectare (1998) to 6,64 t/hectare (2011). Within five years received low productivity (1,58-2,35 t/hectare), and within 11 years average (2,89-4,16 t/hectare) and 9 years high (4,80-6,69 t/hectare).

Studying of correlative dependence of winter rye exemplars productivity and loss of cages in the main stages of vegetation for all years of the considerable influence of this factor didn't show. The analysis in years with low productivity allowed to establish low negative dependence in autumn ( $r = - 0,36$ ) and high in winter ( $r = - 0,75$ ) vegetation stages. In years with high productivity low negative influence is noted ( $r = - 0,32$ ) in the summer. Positive influence of the sum of precipitations was noted in the spring in years with average ( $r = 0,55$ ) and high productivity ( $r = 0,34$ ).

Definition of influence rainfall sum by months of vegetation showed distinctions in years with the different level of productivity. In years with low productivity it is established a high negative correlation with loss of rainfall in October ( $r = - 0,89$ ), November ( $r = - 0,53$ ), February ( $r = - 0,87$ ), May ( $r = - 0,48$ ) and June ( $r = - 0,92$ ). In years with average productivity rainfalls had positive effect in January, February, April and May ( $r = 0,34-0,56$ ), the negative influence it is not revealed. In years with high productivity impact of rainfall was expressed much more weakly as the negative impact of loss of rainfall in October, November, January and March ( $r = -0,30-0,49$ ), positive in December ( $r = 0,47$ ) is established.

As a result of researches concluded that in years with low productivity of a winter rye factors of security with heat and moisture were the main among what created adverse conditions for formation of efficiency of plants.

# Susceptibility of rye genotypes to ergot in artificial inoculation conditions

poster s6-08

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Most of the worldwide rye (*Secale cereale* L.) is produced in Europe, and within the EU Germany and Poland are the largest producers. They harvested on a total acreage of 1.7 Mio hectares 5.1 million tonnes in 2011 (FAOSTAT 2013), this is more than 80% of the total rye production of the EU.

Ergot, caused by the fungus *Claviceps purpurea*, is a severe disease in rye (*Secale cereale* L.) leading to purplish-black sclerotia in the ear that contain >30 mycotoxins, ergot alkaloids.

EU threshold levels are at the moment 0.1% of sclerotia or sclerotia fragments in grain for animal nutrition and 0.05% for human consumption. Main problem is that rye is especially prone to ergot infection because of its outcrossing nature. Self-pollinated cereals, like wheat, barley, are much less infected by the ergot fungus. Physiological resistance alone, however, is not enough in this special case, the cultivars should also have a high amount of pollen shedding, because the fungus can only infect open flowers and the flowers of rye stay longer open the less pollen is available. Population cultivars in rye usually display full fertility and are less prone to ergot infection although, due to the outcrossing nature of rye, much more sensitive than e.g. wheat. Hybrid cultivars are based on cytoplasmically male sterility (CMS) and may contain fully or partially male-sterile single plants in varying percentages due to incomplete pollen-fertility restoration

The aim of three years field trials carried out at two places (Kościelna Wieś, Zybiszów) was to test a special experimental design with artificial inoculation of ergot in order to evaluate susceptibility of rye genotypes to ergot.

In the experiments wide differentiation in ergot infection on individual genotypes was observed. In the 2011/12 season KWS EXP Hybrid II was the most resistant genotype. This genotype contains restorer genes which are responsible for the production of large amounts of pollen during flowering. KWS EXP Hybrid I was the most infected by ergot, which was connected with the lack of effective restorer genes. In the 2012/13 season most resistant genotypes were KWS EXP Hybrid I and KWS Hybrid EXP IV, whereas KWS Hybrid III EXP, KWS EXP Hybrid II and variety SU Allawi (10-percent of the population admixture) were the most infected. In the third year of the experiment at Kościelna Wieś significantly higher ergot infection was observed than at Zybiszów. Strong lodging of rye genotypes was observed at Zybiszów and the results of the experiment were not analyzed. Among the untreated genotypes at Kościelna Wieś the most infected were KWS EXP Hybrid IV, HYBRO 01 and HYBRO 02. Under the conditions of artificial inoculation genotypes KWS EXP Hybrid III, Stakatto and HYBRO 03 showed the lowest resistance to the infection by *Claviceps purpurea*.

# **Nutritional and technological quality**

(Session 7)

# Baking qualities of grain hybrids of winter rye

poster s7-01

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Researches conducted in the conditions of forest-steppe of the Republic Bashkortostan showed that hybrids F1 of winter rye of KWS LOCHOW GMBH selection form grain with a high falling number (257-307 s). The falling number of hybrids, and of a population variety, is a changeable indicator of grain quality that is mainly influenced by weather conditions during the period of grain formation.

The content of water-soluble pentosan in the grain of hybrids differs a little (on  $-0,12 \dots +0,22\%$ ) from the value of this indicator of population variety, Chulpan 7. The highest content of water-soluble pentosan is in grain hybrid Guttino (2,67%). The content of water-soluble pentosan in grain hybrids significantly depends on external conditions of plants vegetation (from 2,49 to 2,82%) and decreases with increasing mass of 1000 grains ( $r = -0,546$ ).

The kinematic viscosity of water extract of hybrids grain of winter rye is high enough (47,34-69,18 cCt) and not lower than the population variety Chulpan 7. Grain hybrids Visello (60,39 cCt) and Guttino (69,18 cCt) have relatively high viscosity of water extract.

The quality of bread of grain of rye hybrids is high. The dimensional stability of the bread is estimated as excellent (0,63-0,85) and it significantly depends on the content of water-soluble pentosan ( $r = 0,671$ ) and the viscosity of water extract of grain ( $r = 0,598$ ).

The volume of bread (154-185 cm<sup>3</sup> per 100 g of flour) from grain hybrids of winter rye is at the level or more than variety Chulpan 7 (158 cm<sup>3</sup>). The hybrid Visello has the highest value of this indicator (185 cm<sup>3</sup>). Water-soluble pentosan in grain has some negative impact on bread volume.

The quality of bread of grain hybrid of winter rye and variety Chulpan 7 is somewhat different on a number of organoleptic characteristics. The bread from grain hybrid Picasso has a smooth crust surface, without large cracks and tears, as well as variety Chulpan 7. The crust color of bread at hybrid Picasso was dark brown, and at variety Chulpan 7 and other hybrids varied from light brown to dark brown. The porosity of bread at hybrids Visello and Brasetto is uniform with emptiness, at hybrids Palazzo and Guttino is rather uniform, but with emptiness. The elasticity of bread crumb was characterized as good in the complete absence of residual deformations at hybrids Visello, Brasetto, Palazzo and Guttino, and as medium at hybrid Picasso and variety Chulpan 7 owing to the presence of insignificant residual deformation.

# Ascorbate peroxidase responses to the aluminum toxicity in the rye cultivars with various level of Al – tolerance

poster s7-02

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The soil acidification and the resulting Al toxicity represents one of the most severe environmental factors limiting the crop productivity. The Al-containing compounds in acidic soils are being solubilized into most phytotoxic  $Al^{3+}$  ions and released into the soil solution where it can cause root stunting, thus restricting the ability of crops to acquire water and nutrients. Al toxicity primarily affects cell division and elongation in the root apex. The  $Al^{3+}$  ions penetrating roots, bind to the negative charges of the plasma membrane phospholipids, leading to rigidification and disruption of the membrane. Higher plants evolved two main protective mechanisms in response to the Al stress. The first is an exclusion mechanism in which Al is prevented from moving through the plasma membrane to the cytoplasm in the root cells. This is achieved by the secretion of organic acids from the radical apex to the rhizosphere which, in turn, modifies the pH and chelates the toxic  $Al^{3+}$ . The second mechanism involves chelation of the Al ions by specific proteins, short-chain organic acids, phenolic compounds and tannins, that can bind and form complexes with  $Al^{3+}$ , and their subsequent compartmentation in the vacuole, thus reducing the Al-toxicity in the cell. The effects of various environmental stresses in plants are also known to be mediated, at least partially, by an enhanced generation of the reactive oxygen species (ROS) under stress. Therefore, Al-tolerant plants, in addition to being able to detoxify aluminium, should also have an efficient antioxidative system for effective removal of the ROS.

Recent research focused on the antioxidant enzymes responses to aluminium toxicity. The specific aim of the current work was determination of the ascorbate peroxidase (APX) gene transcription level combined with the APX enzymatic assays under Al stress, with this being studied in two rye cultivars – Bosmo and Brasetto – differing in response to aluminum stress. The relative levels of tested gene were determined by RT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and elongation factor (EF) were used as the reference genes. The RNA were isolated from a seven-day seedlings incubated in the solutions containing 0 (control), 25, 50 and 100 ppm. The incubation times of 4, 8 and 12 hours were applied. The studies revealed various changes in the gene expression in two cultivars – more dynamic in cv Brasetto. Expression in root was lower than in the leaf for all variants. The level of expression in the more tolerant Brasetto, after 12 h of incubation in all concentrations tested was 30% lower compare to the control values. In Bosmo a significant reduction in the gene expression (by 35-40% after 12 hours) was observed at 25 and 50 ppm Al, and a less-defined decrease (by 10%) at the concentration of 100 ppm. In the leaf tissue an increase in expression of both forms of APX Bosmo (15%) and Brasetto (9%) was measured after 12 hours of incubation in a solution supplemented with 50 ppm Al. By contrast, at the highest aluminium level tested the responses were different. The increase in the expression by 160% was observed in Bosmo, whereas in Brasetto decrease in expression

was 45%. There was no clear dose-dependence observed in the expression level in the either variety. The enzymatic assays revealed remarkable differences in the constitutive level of APX – the activity of the peroxidase determined in control plants was distinctly higher in Brasetto as compared to Bosmo. Furthermore, aluminium treatment provoked an evident increase in the enzymatic activity in both cultivars, with the absolute rise being similar for Brasetto and Bosmo. Importantly however, while the high levels of APX activity measured in the former persisted to the end of the experiment, in the later the activities declined with time and increasing aluminium level.

# Status of research and possibility winter rye breeding to improve the baking and food value of grain

poster s7-03

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The parameters of grain quality due to a large group of linked genes, their expression is subject to the significant influence of genetic and environmental factors and their interaction. Therefore, the study of features formation of technological properties in different growth conditions is very important for adaptive selection.

The methods used for the biochemical and baking indices are 1. Hagberg Falling Number (FN) – ICC 107/1 (1995) method; 2. Amylograph – ICC 126/1 (1992) method; 3. Total pentosans measurements, water soluble pentosans content – Hashimoto method modified by Delcour (1989); 4. kinematic viscosity of water extract of the rye – method advanced by Boros D. et al. (1993), glass capillary viscometer 1.52 Labtex ISO 3104-84.

Over the 20-year period, the variability of quality traits characterizes very high effect of conditions and ranked in descending order as follows: Falling Number (FN) - 92.9%, peak temperature of gelatinization – 83.4%, full-scale grain weight - 68.3%, protein content – 63.7%, viscosity - 62.6%. The most reliable indicators for selection are mass 1000 kernels (MTK) and uniformity of grain, whose share of the effect of genotype was significant (57% and 69%, respectively).

Correlation «FN – protein content» was no significant ( $r = 0,072$ ). Specific volume of bread (ratio of the height/diameter) correlates positively with FN ( $r = 0,774$ ), height amylograph ( $r = 0,856$ ), the gelatinization peak temperature ( $r = 0,750$ ). Was obtained moderate negative correlation between viscosity of water extract of grain meal and MTK ( $r = -0,522$ ) and uniformity of grain ( $r = -0,624$ ).

Long-term analysis of varieties of rye competitive trials revealed a positive correlation between kinematic viscosity and amylograph ( $r = 0,621$ ) and FN ( $r = 0,471$ ). However, significant association between Falling Number, height amylograph and viscosity of water extract of grain meal was observed only in some years or has not been.

In years with extremely hot conditions of high water absorption capacity pentosans increased hydrodynamic properties of swelling and viscosity of water extract, resulting in increased index kinematic viscosity of extracts from grain meal (as well as FN, height amylograph). The high estimates do not correspond to objective reality quality. In excessively wet years, the action of the enzymes present in the rye, degrade the viscous properties of pentosans and reduce their ability swelling and water retention capacity. Therefore, the most favorable years for breeding kinematic viscosity are the years when there is an optimum or medium dry weather conditions during the grain filling.

The share of influence of the genotype on the viscosity was 25.6%, whereas environmental factors accounted for 49.8% of the variance of this trait. The interaction genotype-year" was also high (24.5%). Strong variability of this trait makes a reliable estimate of the genotype to phenotype during breeding. This can significantly lengthen the time to create special purpose of varieties or complicate selection process more expensive analyses.

# Technological properties of winter rye corn

poster s7-04

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Winter rye cultivation in the Volga region is of great importance due to its drought resistance and stability to produce crops. In 2014, this cereal was sown on 158,893 hectares in the Saratov region (16% of the winter crop area). To study the technological properties of rye grains is necessary in connection with the general directions of use: from baking bread to feed production.

The object of our research was several varieties of the crop collection of VIR in 2007 and 2009; the widespread varieties Saratovskaya 7, Marusenka; and new, different light yellow color grain varieties Memory Bambysheva and Sunny.

In 2007, these varieties were characterized by high values of the weight of 1,000 seeds and falling numbers: SMN-16, D. Troubsko (Poland), Kaupo (Latvia), Haru 4 (Japan), Heines Hellnoru, Perkow (Germany), Sentinel (UK), and others. According to the grain size they are much inferior to the standard Saratovskaya 7 ( $m_{1000} = 35.2$  g) and falling number of its superior (210). In the context of 2009, the studied varieties were characterized by a high number of downs, but it varied considerably in size, nature grain and the rheological properties of aqueous suspensions based on meal. In terms of VS30 we have identified 4 groups of varieties, which are characterized by the following values: 1 - 200-385; 2 - 385-570; 3 - 570-755; 4 - 755-940 (EV). The first group is best suited for the production of animal feed, the second group is for baking bread, and the third and fourth ones are used as components in the preparation of feed. In breeding for improving the baking properties of the most promising varieties that combine high viscosity to kind of grain more than 700 g / L and a mass of 1000 seeds, close to 30.0 g: SCW 1304, Wibro, Motto, Jaiton, Perkow, Heines Hellnoru, IN - 14, Stran Liroge, Duntus.

Our study of the varieties of the Saratov winter rye selection in 2013-2014 revealed that they form filled grains with a good vitreous, high full-scale mass. Their amylograms height ranges from 350 to 650 EA, which is characteristic of flour for quality baking. By the slurry viscosity, they fall into the first group. The grain variety Sunny is recommended for the use as a component of animal feed. The grain varieties Saratovskaya 7, Marusenka, and Memory Bambysheva are recommended for the use in baking and fodder production.

# Results of pigs feeding by grain forage winter rye of cultivar Yantarnaya

poster s7-05

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In 2011 year the work began in FSBSI “Uralsky NIISH” at create a new cultivar of winter rye for feed directions of use under supervision professor Doctor biological Science Kobylansky V. D. The cultivar of winter rye Yantarnaya was created on base material was derived from Kobylansky V. D. The cultivar of winter rye Yantarnaya is the first on Ural in new cultivars group with low content water-soluble pentosans. The cultivar have been passing The State test from 2014 year.

The reconnaissance test were passed in 2013 year in FSBSI “Uralsky NIISH” under by feeding pigs. Results this test with change in ration 20 % grain of wheat on 20 % grain of rye new cultivar Yantarnaya were showed high palatability and nutrient quality of feed. In test group feed required 33.9 kg per unit kg of gain that higher to 8.2 % than control group. In control group, feed consumption was 3.69 kg. Average daily gain was 730 g in control group and 720 g in test group that was lower on 1.4 % it was in limits experimental error. The general feed consumption (114.3 kg) was reduced per head of livestock in test group to 11.5 % in compared with control group (129.1 kg). The feed cost per one-kilo weight gain in monetary equivalent reduced on 8.2 % with uses winter rye of low content water-soluble pentosans.

The experiment carried in pig-breeding complex “Gornouralsky” to two groups in 2014 year. In every groups had 100 pigs. It showed that if 10 % of grain of wheat and 10 % of grain of barley to change on 20 % of grain of winter rye of cultivar Yantarnaya in feed for pigs fed good ate, pigs did not sick, but significant gain or decrease did not determine and meat yield of primary grade gain on 1.2 % and secondary grade gain on 4.7 %.

The cultivar of winter rye Yantarnaya may include in composition fodder for animals.

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