

INTERNATIONAL SYMPOSIUM  
ON RYE BREEDING & GENETICS

**RY**  **EST**  
**2025**

17-19 June 2025 in Tartu, Estonia

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**ABSTRACT  
BOOK**

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## Dear colleagues and rye friends,

On behalf of the RYEST2025 Organizing Committee, we warmly welcome you to the International Symposium on Rye Breeding and Genetics in the frame of the EUCARPIA Cereals Section. We are delighted to host this event in the academic heart of Estonia – Tartu.

This year, we proudly dedicate the symposium to one of the oldest winter rye varieties, 'Sangaste', as we celebrate its 150th anniversary. Since its development, the field of plant breeding and cultivation has undergone remarkable transformations. Today's breeders and researchers face new challenges driven by evolving goals and changing environmental conditions. Technological advancements have been extraordinary. We now understand rye genetics more deeply and apply this knowledge to modern breeding strategies. The integration of cutting-edge research, including Artificial Intelligence, continues to expand our capabilities and vision for the future.

RYEST2025 serves as a vibrant platform for researchers to exchange ideas, explore novel opportunities, and reconnect with colleagues—both new and familiar. The impressive scientific programme comprises 28 lectures, including 10 keynote lectures, and 16 posters presented in five sessions. To combine science and historical view a visit to the METK's breeding station in Jõgeva and the birth place of 'Sangaste' is planned in the programme. We want to thank all sponsors who have supported the successful organisation of the conference.

We extend our heartfelt thanks to all authors, participants, and members of the scientific and organizing committees. We hope all delegates enjoy their time in Tartu, Estonia, and leave inspired by the shared knowledge and collaboration.

***Dr. Liina Jakobson (METK)***

***Dr. Kadri Sohar (METK)***

## Sponsors

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## Programme

Tuesday, 17 June

- 08:00-09:00 Registration  
09:00-09:10 **Andreas Börner** Opening Remarks on behalf EUCARPIA  
09:10-09:20 **Viktor Korzun** Opening Remarks on behalf EUCARPIA RYE Group  
09:20-09:30 **Marko Kass** Opening Remarks on behalf of METK (Centre of Estonian Rural Research and Knowledge)

### SESSION I RYE GENETIC RESOURCES AND DIVERSITY Chairs: Andreas Börner & Evelin Loit-Harro

- 09:30-10:00 KEYNOTE LECTURE  
**Nils Stein** *The rye pangenome – do you know your germplasm?*  
10:00-10:15 **Maja Boczkowska** *Conservation, characterization, and genetic analysis of Polish rye genetic resources*  
10:15-10:30 **Steven Dreissig** *Pollen and anther morphological variation in rye was shaped by domestication*  
10:30-10:45 **Dörthe Siekmann** *From evolution to innovation – Breeding rye for a sustainable bioeconomy*  
10:45-11:15 Coffee Break

### SESSION II RYE BREEDING METHODS AND MOLECULAR TOOLS Chairs: Viktor Korzun & Liina Jakobson

- 11:15-11:45 KEYNOTE LECTURE  
**Mark Sorrells** *Plant Breeding in the 21st Century: Molecular Breeding and High Throughput Phenotyping*  
11:45-12:15 KEYNOTE LECTURE  
**Pernille Merete Sarup** *Genomic prediction of general combining ability and specific combining ability in 3-way rye crosses*  
12:15-12:30 **Marvin Rose** *Enhancing selection gain of population breeding in winter rye by using genomic selection*  
12:30-13:30 Lunch Break  
13:30-14:00 KEYNOTE LECTURE  
**Andres Gordillo** *Genomic selection in hybrid rye breeding: from theory to practice*  
14:00-14:15 **Peter Skov Kristensen** *Optimizing hybrid rye breeding programs using simulations*  
14:15-15:15 Elevator Pitches/Meet the Sponsors/Poster session I  
15:15-15:45 Coffee Break

**Chairs: Mark Sorrells & Pernille Merete Sarup**

- 15:45-16:15 KEYNOTE LECTURE  
**Stefan Stojalowski** *Thirty years of molecular markers for CMS systems in rye – a subjective review*  
16:15-16:30 **Kadri Sohar** *Testing spring rye cultivation in Estonia*  
16:30-16:45 **Christina Waesch** *Influence of long-term nutrient deficiency on pollen and anther morphological traits in rye*  
16:45-17:10 **Bernd Hackauf** *RYE2050: A vision for evolutionary rye breeding*  
19:00-21:00 Welcome reception at the University of Tartu Delta Centre

## Wednesday, 18 June

### SESSION III RYE DISEASE RESISTANCE AND STRESS TOLERANCE

**Chairs: Stefan Stojalowski & Heinrich Gausgruber**

- 09:00-09:30 KEYNOTE LECTURE  
**Thomas Miedaner** *Breeding for fungal disease resistances in small-grain cereals*
- 09:30-09:45 **Khalid Mahmood** *Rare missense mutations in the C terminal and NB-ARC domain of pm3 like disease resistance protein confer powdery mildew disease resistance in rye*
- 09:45-10:00 **David Chan-Rodriguez** *Comparative transcriptomics of rye (*Secale cereale* L.) inbred lines revealed candidate genes involved in phosphorus deficiency*
- 10:00-10:15 **Bernd Hackauf** *Impact of the GA-sensitive dwarfing gene Ddw1 on the performance of rye under drought*
- 10:15-10:30 **Pawel Dopierala** *Viral diseases transmitted by insects in rye*
- 10:30-11:00 Coffee Break
- 11:00-12:00 Elevator Pitches/Meet the Sponsors/Poster session II
- 12:00-13:00 Lunch Break

### SESSION IV RYE FOR FOOD, FEED, FUEL AND FUN

**Chairs: Thomas Miedaner & Kadri Sohar**

- 13:00-13:30 KEYNOTE LECTURE  
**Heinrich Gausgruber** *Comin' thro' the rye*
- 13:30-13:45 **Lisa Kissing Kucek** *Breeding cereal rye for cover crop and forage in the United States*
- 13:45-14:00 **Virginia Nichols** *Partially perennial? Self-cover cropping cereal rye as a novel application for material produced from perennial grain breeding efforts*
- 14:00-14:15 **Anna Szafrńska** *Investigating the variability of free asparagine content in rye cultivars grown in Poland*
- 14:15-14:30 **Evelin Loit-Harro** *Rye as a Valuable Cover Crop*
- 14:30-14:45 Group Photo
- 14:45-15:00 Coffee Break

### SESSION V PAST & FUTURE OF RYE RESEARCH AND BREEDING

**Chairs: Andreas Börner & Teija Tenhola-Roininen**

- 15:00-15:30 KEYNOTE LECTURE  
**Jochen Kumlehn** *Genome editing in rye – lessons from other Triticeae cereals*
- 15:30-16:00 KEYNOTE LECTURE  
**Henryk Bujak** *Winter rye breeding progress-where do we stand?*
- 16:00-16:15 **Jacob Eifler** *History of KWS short hybrid rye: Applied product innovation and its value for farmers*
- 16:15-16:30 **Mauricio Carlos Kuki** *New perspectives for rye: Facultative hybrids*
- 16:30-17:00 KEYNOTE LECTURE  
**Viktor Korzun** *Rye research and breeding: looking back and forward*
- 17:00-17:15 Updates and Announcement Regarding the Next Conference
- 17:15-17:30 Closing Remarks
- 19:00-23:00 Symposium dinner at Vilde ja Vine restaurant

## Thursday, 19 June

### Field trip & Sangaste visit

- 08:30-09:30 Bus travel to Jõgeva
- 09:30-12:30 RYEST field trial and visiting METK campus
- 12:30-14:30 Bus travel to Sangaste
- 14:30-17:00 Sangaste castle – the birthplace of “Sangaste” rye
- 17:00-18:00 Bus travel to Tartu

# SESSION I

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## **RYE GENETIC RESOURCES AND DIVERSITY**

## The rye pangenome – do you know your germplasm?

### **Nils Stein**

*Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Seeland, Germany*

The genomic revolution, initiated by the introduction of high-throughput next generation sequencing technology combined with new compute power and algorithms, has made *de novo* sequencing and assembly a routine task, almost independent of genome complexity. While sequencing of the multigigabase genomes of the Triticeae crop species barley, wheat, rye initially required the collaboration of larger research consortia, sequencing entire Triticeae genomes has just become a routine task and is easily implemented as part of a map-based cloning project. This opens the perspective towards systematically unlocking the crop genome diversity at species scale and in barley this has allowed to capture global barley genome diversity by shallow reduced representation sequencing (GBS) of 40,000 accessions from different genebanks as well as by chromosome-scale genome assembly of more than 150 genotypes. Rye has also a diploid but an almost 50% larger genome than barley and the cross-breeding nature of rye leads to high heterozygosity, which cannot easily be reduced through selfing because of self-incompatibility and inbreeding depression. Two whole genome assemblies were published in rye, both providing important resources to rye research and breeding but both are still of limited quality, hence there is still a desperate need to improve rye genomic resources. Progress will be presented towards high-quality chromosome-scale genome assembly and also towards phased diploid genome assembly in rye and how these developments open the perspective for pangenome analysis in this important Northern European crop species.

## Conservation, Characterization, and Genetic Analysis of Polish Rye Genetic Resources

### **Maja Boczkowska**

*National Center for Plant Genetic Resources, Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, 05-870 Błonie, Poland*

Poland has a long-standing tradition in rye (*Secale cereale* L.) cultivation, resulting in a considerable diversity of genetic resources adapted to various environmental conditions. The National Center for Plant Genetic Resources (NCPGR), operating at the Plant Breeding and Acclimatization Institute – National Research Institute (IHAR-PIB) in Radzików, plays a critical role in conserving and characterizing Polish rye gene resources. The National Gene Bank maintains a comprehensive collection, including landraces, breeding lines, traditional cultivars, and wild relatives, representing valuable genetic variability crucial for future breeding programs and sustainable agriculture.

The rye collection at NCPGR currently comprises approximately 2,700 accessions, systematically preserved ex-situ in cold-storage facilities to ensure long-term viability. Each accession undergoes thorough characterization and evaluation, encompassing morphological traits, agronomic performance, disease resistance, and abiotic stress tolerance. Additionally, molecular characterization utilizing NGS-based DArTseq markers significantly enhances understanding genetic relationships, population structure, and diversity among accessions.

DArTseq combines genome complexity reduction and next-generation sequencing to identify thousands of genome-wide single nucleotide polymorphisms (SNPs). This approach enables efficient, cost-effective, and precise characterization of rye germplasm at the genomic level, significantly improving genetic resource management, trait mapping, and selection of promising parental genotypes for breeding.

The Polish rye genetic resources preserved at NCPGR harbor valuable adaptive traits, including winter hardiness, drought tolerance, and efficient nutrient use, which are increasingly important under climate change scenarios. So far, Polish and Turkish landraces have been genotyped using DArTseq. Notably, local landraces often carry unique alleles absent from modern varieties, thus representing an irreplaceable genetic reservoir for future breeding initiatives.

## Pollen and anther morphological variation in rye was shaped by domestication

**Christina Waesch<sup>1</sup>, Yixuan Gao<sup>1</sup>, Natalie Koch<sup>1</sup>, Christin-Sophie Gaede<sup>1</sup>, Thomas Hornick<sup>2,3</sup>, Christian Dusny<sup>2</sup>, Jörg Fuchs<sup>4</sup>, Andreas Börner<sup>4</sup>, Axel Himmelbach<sup>4</sup>, Martin Mascher<sup>4,3</sup>, Klaus Pillen<sup>1</sup>, Susanne Dunker<sup>2,3</sup>, Steven Dreissig<sup>1,3,4\*</sup>**

<sup>1</sup> Institute of Agricultural and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

<sup>2</sup> Helmholtz-Centre for Environmental Research – UFZ Leipzig, Leipzig, Germany

<sup>3</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

<sup>4</sup> Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, OT Gatersleben, Germany

In plants and animals, pollen or sperm morphology differ greatly between species. Across plant species, pollen morphological diversity is broadly linked to different pollination systems. However, the extent of within-species diversity is less well understood. To address this question, we explored pollen and anther diversity in rye (*Secale cereale* L.), a wind-pollinating grass species.

We analysed 339 domesticated, feral and wild rye individuals of 64 diverse accessions. Population structure analysis revealed a differentiation gradient from wild to domesticated rye. We found pronounced within-species diversity of pollen and anther morphology. Genome-wide association scans uncovered a polygenic architecture of pollen and anther traits, with medium to high heritability and mostly small-effect loci. A subset of these loci overlapped with previously identified domestication loci, for which the underlying traits were unknown. A  $P_{ST}$ - $F_{ST}$  analysis suggests that pollen and anther traits were under selection throughout rye domestication. Population genomic analyses revealed signatures of selection at 37 % of all identified loci.

Our work shows that selection for larger pollen grains and longer anthers occurred throughout rye domestication. The present study extends our knowledge of the genetic architecture underlying within-species pollen and anther morphological diversity, and further unravels domestication traits in rye.

## From Evolution to Innovation – Breeding Rye for a Sustainable Bioeconomy

**Dörthe Siekmann<sup>1</sup> and Bernd Hackauf<sup>2</sup>**

<sup>1</sup>*HYBRO Saatzucht GmbH & Co. KG, 17291 Schenkenberg, Germany*

<sup>2</sup>*Julius Kühn Institute, 18190 Groß Lüsewitz, Germany*

Resilience through crop diversification is a key element in sustainable intensification of agriculture to satisfy the increasing demand for food of a growing human population under climate change. A stringent diversification of cereal crop rotations is part of this, and rye (*Secale cereale* L.) offers strong potential in terms of the modernization of our primary production systems, environmental protection, and the maintenance and restoration of biodiversity. Considering 20% less greenhouse gas emissions and 8% lower Carbon Footprint as compared to wheat [1], an increase of the cultivation area of hybrid rye provides a viable mitigation option in line with the *European Green Deal* to transform the EU into a sustainable, resource-efficient, and competitive bioeconomy with zero net emissions of greenhouse gases by 2050. We introduce the interdisciplinary and pre-competitive research project RYE-HUB that aims to advance the development of semi-dwarf rye hybrids by i.) identification of the gene and allele repertoire of rye through establishing a pan-genome sequence, ii.) a comprehensive characterization of natural diversity patterns captured from rye germplasm resources using precision phenotyping, next-generation sequencing and population genetic methods as a prerequisite for the identification of valuable alleles, iii.) understanding the genetics of complex agronomic and qualitative traits based on joint linkage and association mapping of quantitative trait loci (QTL) in rye, iv.) establishing doubled haploid technology in rye to considerably shorten the length of a breeding cycle, and v.) expanding the catalogue of functionally characterized genes in rye. For this purpose, we have started to develop a large-scale rye Nested-Association Mapping (NAM) population, aiming to establish 6,000 recombinant inbred lines derived from the crosses of a sequenced self-fertile common parent [2] with each of 60 diverse founders. A large set of rye recombinant inbred lines in the public domain will allow a wide range of scientists to integrate their research together in community efforts and community databases as trigger for more attention to rye.

**Acknowledgement:** The project RYE-HUB receives funding by the German Federal Ministry of Education and Research (Grant No. 031B1541 A-G)

[1] Riedesel L., Laidig F., Hadasch S., Rentel D., Hackauf B., Piepho H.-P., Feike T. Breeding progress reduces carbon footprints of wheat and rye. *J. Clean. Prod.* 377, 134326 (2022)

[2] Rabanus-Wallace M.T., Hackauf B., Mascher M., ..., Stein N. Chromosome-scale genome assembly provides insights into rye biology, evolution, and agronomic potential. *Nat. Genet.* 53: 564-573 (2021)

## Root physiological and architectural characteristics of semi-dwarf rye

**Md Mohsin Morad<sup>1</sup>, Jakob Eifler<sup>2</sup>, Morten Sahr<sup>2</sup>, Nicolaus von Wirén<sup>1</sup>, Ricardo F.H. Giehl<sup>1</sup>**

<sup>1</sup> Leibniz Institute of Plant Genetics & Crop Plant Research (IPK) OT Gatersleben, Corrensstr. 3, 06466 Seeland, Germany

<sup>2</sup> KWS LOCHOW GMBH, Ferdinand-von-Lochow-Straße 5, 29303 Bergen, Germany

The reduction of stem length by dwarfing gene alleles has been exploited in plant breeding to improve harvest index, increase lodging resistance, and boost grain yield. Compared to other small grain cereals, deployment of semi-dwarfism in rye is still less developed. A promising strategy to reduce the height of rye is the introduction of the dominant, gibberellin-sensitive dwarfing gene *Ddw1*. Though *Ddw1* can significantly decrease shoot stature, whether it affects root system architecture, rooting depth and nitrogen (N) uptake capacity has not been extensively investigated. In this study, we used rhizoboxes, soil-filled columns and hydroponics to assess several physiological and architectural root traits of semi-dwarf and tall rye inbred lines, alongside winter rye hybrids. At the seedling stage, rye hybrids had larger root systems compared to lines, with no significant reduction in total root length was observed between tall and semi-dwarf genotypes. In hydroponics, semi-dwarf hybrids had comparable rooting depth (length of longest axial root) to their corresponding tall hybrid genotype. Root mass distribution in different soil profiles was further investigated by growing vernalized plants in soil-filled columns until start of flowering. In this system, hybrid genotypes exhibited larger root biomass than the inbred lines, in agreement with the results obtained at the seedling stage in rhizoboxes. Compared to lines, hybrids had on average two times more root mass at depths of 100 cm to 150 cm. Finally, we assessed the performance of the hybrids under N-limited conditions. Introduction of *Ddw1* did not negatively affect the capacity of plants to stimulate root growth in response to low N. Furthermore, shoot and root N concentration as well as root N uptake capacity of semi-dwarf hybrids was indistinguishable from the corresponding tall hybrid. Together, our results indicate that the introduction of *Ddw1* in winter rye mainly impacted shoot traits, with relatively minor effects on the function, growth and architecture of root systems, especially in hybrids.

## NordGen Rye Collection: Conserving Rye Genetic Diversity for Future Research and Breeding

**Michael F. Lyngkjær<sup>1</sup> & Jan Svensso<sup>1</sup>**

<sup>1</sup>NordGen - Nordic Genetic Resource Center, Växthusvägen 12, 234 56 Alnarp, Sweden

NordGen maintains a diverse and strategically important rye (*Secale cereale*) collection, comprising 390 active accessions vital for future breeding and research programs. The collection predominantly represents Northern European origins, with significant contributions from Sweden (187 accessions) and Finland (149 accessions), supplemented by smaller yet essential representations from Norway (11), Germany (11), and various other regions including Afghanistan, Poland, Russia, Denmark, Estonia, Latvia, China, Turkey, and Bulgaria.

This collection encompasses a broad spectrum of genetic diversity across different improvement levels, including 76 advanced or improved cultivars, 147 traditional cultivars or landraces, and 157 accessions categorized as breeding or research materials. This diversity supports exploration into key adaptation traits, genetic resilience, and quality attributes necessary to address challenges in agriculture and climate variability.

Historical accession records date as far back as 1875, reflecting significant breeding milestones throughout the early to mid-20th century. Previous agronomic evaluations within the NordGen rye collection have focused on traits critical for breeding of spring rye, such as lodging resistance, plant height, time of ear emergence, ear length, and density. Conducted under varying Nordic environmental conditions, these evaluations offer valuable initial insights into the agronomic performance of rye accessions, emphasizing the need for ongoing, comprehensive characterization.

Overall, NordGen's rye collection represents a valuable genetic resource crucial for sustainable agricultural practices, enhancing crop resilience, and supporting future breeding objectives regionally and globally.

## Genetic Diversity of Wild and Cultivated Rye Revealed by Low Pass WGS Derived SNP Markers

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Rye (*Secale cereale* L.) is a climate-resilient cereal crop widely used in the development of improved wheat varieties and equipped with a complete set of genes essential for hybrid breeding [1]. Winter rye, closely related to wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), is predominantly cultivated for human consumption and livestock feed and is recognized for its ability to achieve high yields even under challenging environmental conditions. Its nutrient efficiency and disease tolerance reduce the need for fertilizers and pesticides and lead to increased consumer interest in rye-based products [2].

As an outcrossing species, rye maintains access to a rich pool of genetic diversity from its wild relatives [1]. This genetic reservoir holds significant potential for advancing rye breeding programs and offers opportunities to address the challenges of incorporating rye traits into wheat improvement initiatives. However, the distribution of genetic diversity among subspecies and across different geographic regions remains poorly understood [3].

Progress in rye breeding still remains slow due to the challenges associated with its allogamy nature, self-incompatibility, and susceptibility to inbreeding depression [2]. Advances in molecular breeding for rye depend on the availability of high-density molecular linkage maps. This work investigates the genetic diversity structure of wild and cultivated rye populations. The rye material, obtained from IHAR Gene Bank (Poland), included 40 accessions of historical and modern cultivars, as well as wild varieties representing a wide geographical diversity originating from Poland, Ukraine, Azerbaijan and other countries. To reveal the genetic diversity structure, SNP markers were obtained using the Low Pass WGS method. DNA sequencing and Variant Calling analysis were performed to detect these markers. Genetic diversity was established using a distance-based method on a matrix of genotyped SNPs. Additionally, a dendrogram and cluster analysis were performed, along with multidimensional scaling (MDS). Structure analysis using STRUCTURE software was also conducted, revealing a large differentiation within the analysed population.

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## Identification of sources of genetic variation suitable for extension of the genetic base of heterotic pools used for rye hybrid breeding in Poland

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Hybrid rye breeding leads to considerably faster improvement of grain yield and the other functionally and quality important features of varieties in comparison to the traditional population breeding (1). However, to ensure continued genetic gains in hybrid breeding and extend hybrid cultivation to new areas the genetic base of heterotic pools must be constantly powered with new genetic variability (2). The aim of the present study was to identify the sources of genetic variation suitable for extension of the genetic base of heterotic pools used for rye hybrid breeding by two breeding companies from Poland: Poznańska Hodowla Roślin Ltd. and DANKO Hodowla Roślin Ltd.

The basic material comprised six population and six F1 rye varieties: Amilo, Armand, Bosmo, D. Diament, D. Granat, Stanko, Vajtka, Gonello F1, Konto F1, Palazzo F1, Skaltio F1, Stakkato F1, Tur F1, Visello F1 and two sets of reference germplasm included 173 non-restorer lines and 113 restorer type lines. In total 1588 individuals were analyzed. Genetic structure and the level of genetic variation was analyzed using the selected set of 4.5 K SNPs loci which showed high level of heterozygosity in F1 rye hybrid varieties included to the study.

The AMOVA analysis showed that the genetic differentiation between analyzed materials was high and statistically significant with  $F_{ST} = 14.0\%$  ( $P = 0,0001$ ). The non-restorer lines from PHR and DANKO showed the highest genetic similarity to the population rye varieties with average  $F_{ST} = 7.0\%$ , whereas the restorer type lines were the most similar to Visello F1 variety with average  $F_{ST} = 8.5\%$ . The genetic differentiation was also found between two non-restorer groups of lines with  $F_{ST} = 6.5\%$ . The restorer type groups of lines from PHR and DANKO were genetically similar with  $F_{ST} = 2.5\%$ . The Bayesian assignment of samples by STRUCTURE revealed that the overall gene pool of the analyzed materials is represented by two ( $K = 2$ ) genetic clusters. Most of the analyzed individuals (62%) was classified with the probability higher than 70% to the non-restorer group. The restorer group comprise 18% of the studied individuals. The obtained results suggest that the populations varieties are good candidates for introgression in non-restorer gene pool whereas the F1 varieties like Gonello, Palazzo, Skaltio and Stakkato are sources of genetic variation for extension of the genetic base of restorer pool, that the use of which should not reduce of the genetic distance between the established heterotic pools.

## SESSION I: RYE GENETIC RESOURCES AND DIVERSITY

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# SESSION II

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## **RYE BREEDING METHODS AND MOLECULAR TOOLS**

## **Plant Breeding in the 21<sup>st</sup> Century: Molecular Breeding and High Throughput Phenotyping**

**Mark Earl Sorrells**

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The discipline of plant breeding is experiencing a renaissance impacting crop improvement as a result of new technologies, however fundamental questions remain for predicting the phenotype and how the environment and genetics shape it. Inexpensive DNA sequencing, genotyping, new statistical methods, high throughput phenotyping and gene-editing are revolutionizing breeding methods and strategies for improving both quantitative and qualitative traits. Genomic selection (GS) models use genome-wide markers to predict performance for both phenotyped and non-phenotyped individuals. Aerial and ground imaging systems generate data on correlated traits such as canopy temperature and normalized difference vegetative index that can be combined with genotypes in multivariate models to further increase prediction accuracy and reduce the cost of advanced trials with limited replication in time and space. Design of a GS 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 genotype by environment interaction (GxE) effects to identify a highly predictive subset of environments. We have developed a methodology for analyzing unbalanced datasets using genome-wide marker effects to group environments and identify outlier environments. Environmental covariates can be identified using a crop model and used in a GS model to predict GxE in unobserved environments and to predict performance in climate change scenarios. These new tools and knowledge challenge the plant breeder to ask the right questions and choose the tools that are appropriate for their crop and target traits. Contemporary plant breeding requires teams of people with expertise in genetics, physiology, phenotyping and statistics to improve efficiency and increase prediction accuracy in terms of genotypes, experimental design and environment sampling.

## Genomic prediction of general combining ability and specific combining ability in 3-way Rye crosses

**Pernille Merete Sarup**

*Nordic Seed*

Genomic prediction using genomic best unbiased linear prediction (GBLUP) is a tool that is used extensively to predict the additive genetic value in breeding populations of animal and plants. The concept was first established by Meuwissen et al (2001), and the main goal is to predict the genetic value of an individual as a breeding parent. In animal breeding the non-additive genetic value (i.e. the dominance and epistatic genetic value) of an individual is not important, as it is only the additive genetic value that is directly inherited by the offspring, and the offspring is the product. In plant breeding however, the product is a genetically uniform cultivar or hybrid, thus prediction of both additive and non-additive genetic value for the individual genotype becomes crucial for selection of the best cultivar or hybrid.

Rye suffers from inbreeding depression when homozygote and is therefore grown as population cultivars or as hybrid varieties. Hybrids between heterotic groups have markedly higher yield than inbred lines and population cultivars. However, highly inbred hybrid parental lines can pose a problem with low yield in the production of hybrids. The solution to this problem is to keep some heterozygosity in the component lines as well as develop 3-way hybrids that allow for even more heterozygosity (and therefore more yield) on the maternal side in the production. However, models for genomic prediction of additive and non-additive effects within and across different heterotic groups that can account for incomplete inbreeding in hybrid parental lines has been lacking for breeding of hybrid crops. In Kristensen et al (2023) we present and evaluate genomic prediction models accounting for incomplete inbreeding in parental lines from two different heterotic groups. These models can be used for prediction of general combining ability (GCA) of parental lines from each heterotic group as well as specific combining ability (SCA) of all realized and potential crosses. GCA was estimated as the sum of additive genetic effects and within-group epistasis due to a high degree of inbreeding in parental lines. SCA was estimated as the sum of across-group epistasis and dominance effects. All parental components could have any levels of inbreeding. Data from commercial breeding programs for hybrid rye was used to evaluate the models with focus on grain yield. Additive genetic variances were larger than epistatic and dominance variances. The implementation of the genomic prediction models in hybrid breeding programs can potentially lead to increased genetic gain in two different ways: I) by facilitating selection of crossing parents with high GCA within heterotic groups and II) by prediction of SCA of all realized and potential combinations of parental lines to produce hybrids with high total genetic values.

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## SESSION II: RYE BREEDING METHODS AND MOLECULAR TOOLS

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## Enhancing Selection Gain of Population Breeding in Winter Rye by Using Genomic Selection

**Marvin Rose<sup>1</sup>, Paul Gruner<sup>1</sup>, Kim Steige<sup>1</sup>, Thomas Miedaner<sup>1</sup>**

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Winter rye (*Secale cereale* L.) is well suited for organic farming due to its resilience to drought, disease resistance, and efficient nutrient use. The two main variety types, populations (self-incompatible, SI) and hybrids (self-fertile, SF) differ in their flowering biology, leading to distinct breeding approaches. SI complicates phenotypic selection in population breeding, limiting genetic yield improvements compared to hybrids (for grain yield 0.24 vs. 0.77 dt/ha year). Since its introduction in the 1970s, hybrid rye has become the dominant choice in conventional cultivation and breeding. In this project, variety trials under organic farming conditions across three locations showed that hybrids, on average, yielded 23.2% (-5.2% – 42.8%) more than population varieties, confirming that the yield gap remains under organic cultivation. Nevertheless, population varieties remain valuable for organic and low-input farming systems, where the high cost of hybrid seed can be prohibitive. Their diverse genetic structure also enhances adaptability and disease resistance, offering a natural risk diversification strategy.

The *RoggenPop* project aims to revitalize population breeding in winter rye through modern and innovative breeding strategies. To achieve this, our main approach is to use a new breeding scheme utilizing genomic selection for population breeding. Currently, this breeding scheme is applied to two population varieties: Firmament (organic) and Elias (conventional). By training predictive models on genetic and phenotypic datasets from polycross progenies, we enable more precise selection of superior founder genotypes (single plants). The approach leverages genotyping-by-sequencing (GBS) markers and genomic prediction to estimate breeding values of single plants without actual phenotypic data and thus accelerate genetic gain. This enables genomic selection at the single-plant level for yield, allowing selection before another open pollination through the male parent occurs. This strategy provides a novel framework to enhance the competitiveness of population varieties, ensuring their long-term viability in rye cultivation.

## Genomic selection in hybrid rye breeding: from theory to practice

**Andrés Gordillo<sup>1</sup>, Morten Sahr<sup>1</sup> and Rajavel Arumugam<sup>1</sup>**

*<sup>1</sup>KWS LOCHOW GMBH*

Before starting a genomic selection (GS) program, it is essential to understand the genetic structure of the prediction population (PredP) to design the estimation population (EP) accordingly. Prediction accuracy of GS is composed of accuracy of prediction between families (mid-parent values) and accuracy of prediction within families (Mendelian variation). Assessing the advantage of GS over predictions based on mid-parent performance is essential to assess the relative merit of GS [1]. GS schemes can be classified according to the relationship between the EP and PredP, i.e. whether the EP and PredP correspond to the same selection population (GS within selection cycles) or different selection populations (GS across selection cycles). In selection within cycles, phenotypic prediction is somewhat higher than prediction ability of GS for grain yield. Index selection using phenotypic and genomic estimated values is consistently better than phenotypic or genomic selection alone. In GS across selection cycles, the prediction ability for grain yield is considerably lower than for GS within cycles. This confirms the high dependence of prediction ability on the genetic relationship between EP and PredP. Yet, GS across cycles offers the advantage of predicting untested individuals and thus reduce generation intervals and increase genetic gain. The higher the selection intensity based on mid-parent values in early line selection stages prior to selection for general combining ability (GCA), the lower the variance between families and higher the relative portion of Mendelian variation and thus the higher the merit of GS for GCA. Empirical validation experiments with unselected line sets contribute unbiased estimates for the prediction accuracy of phenotypic and genomic selection, which can be used in model calculations to optimally allocate GS resources and maximize genetic gain.

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## Optimizing hybrid rye breeding programs using simulations

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Breeding programs consist of many interacting operations including phenotypic testing and genetic evaluation of potential new varieties for yield, quality, and disease resistance, selection of crossing parents, product development etc. The consequences of changes in breeding programs can often not be evaluated until many years later. Therefore, decision support tools based on stochastic simulations can be used to improve efficiency and find the optimal breeding strategies. Using such tools, *e.g.* ADAM [1], a broad range of complex breeding programs can be simulated from the level of individual genes and genetic markers to phenotypes from different testing systems, and the use of complex statistical models for traits of interest can be evaluated. Thereby, the effects of different breeding strategies on genetic gain and genetic variance over many generations can be investigated.

In this study, breeding schemes for hybrid rye were simulated to compare different genotyping, phenotyping and selection strategies, and thereby investigate the value of testing hybrids as well as parental lines from two different heterotic groups under different assumptions on variance due to additive genetic and dominance/epistatic genetic effects [2]. Genetic gain can be increased by using genomic selection rather than phenotypic selection and by including phenotypes of parental lines in the prediction models.

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2. Chu T.T., Kristensen P.S., Jensen J. Simulation of functional additive and non-additive genetic effects using statistical estimates from quantitative genetic models. *Heredity*, 133, 33-42 (2024). <https://doi.org/10.1038/s41437-024-00690-5>.

## Thirty years of molecular markers for CMS systems in rye – a subjective review

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The first report about cytoplasmic male sterility (CMS) in rye have been published by E. Putt in North America in 1954, but this source of CMS has not been conserved. In the next decade the idea of breeding hybrid cultivars of rye has arisen resulting in several discovered sources of CMS among which the most significant for practice is cytoplasm Pampa reported by Geiger and Schnell in 1970. Numerous alternatives of CMS-Pampa have been discovered at similar times and in following decades. In 1982 Hartwig Geiger suggested that all known non-Pampa sterilizing cytoplasm of rye may be of the same genetic character and proposed the common name CMS-Vavilovii (CMS-V). Over thirty years ago, in 1993 had been published the first molecular marker linked with nuclear fertility restorer gene for Pampa cytoplasm. It was an isozyme marker which allowed localization of studied restorer gene on 1R chromosome [1]. This scientific contribution has been followed by DNA-based markers which allowed for mapping a set of male fertility restorer genes for Pampa cytoplasm on various chromosomes [2] and resulted in application of molecular markers in commercial breeding programs. Few reports concerning molecular markers linked with male sterility restorer genes for cytoplasm of Vavilovii family showed localization of these genes on various chromosomes [3, 4 and other]. In addition, a set of PCR-based molecular markers for identification of the type of cytoplasm was published [5]. The importance of the molecular markers mentioned above for research and for practice will be discussed.

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## Testing spring rye cultivation in Estonia

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Recently, there has been a growing interest in spring rye. It is used as a green fertilizer, green forage, and even grain [1]. Funded by Estonian Ministry of Science and Education (Centre of Excellence AgroCropFuture) new or less known crops for Estonia are tested due to changes in environment and weather conditions. For the project the spring rye is one of the perspective crops.

Ten varieties of spring rye from Germany and Poland were proved in the Centre of Estonian Rural Research and Knowledge (METK) at 2024. Spring rye was sown on May 2nd with sowing rate 350 seeds per m<sup>2</sup> (fertilizer (NPK(S) 300kg/ha, soil pH 6.1). To sum it for tested varieties, sprouting time was ten days. Plants endured a cold and dry spring, the lowest- 5.7°C, precipitation 9 mm (norm 42 mm) in May at Jõgeva [2], and were affected by insect pests (*Phyllotreta* sp.). Thus, the test-field was treated with insecticide.

The summer average air temperature was 17.6°C (norm 16.4°C) and sum of precipitation 280 mm (norm 218 mm) in Jõgeva [2]. Spring rye flowered from late-June to early-July. The average number of productive stalks was 2\_3. Leaf rust was identified on plants. Spring rye lodged easily in trials due to sudden heavy rain showers and windy summer.

The harvesting time of spring rye was on August 14th. The average yield was 1 tons/ha, the thousand grain weight was 26.8 g, hectolitre weight 616 g/l. To conclude, to attain better yield, agrotechnology for spring rye in Estonia should be improved, for example, possible earlier sowing time or increase sowing rate and fertilizer amount.

[1] Klikocka H., Podleśna A., Narolski B., Podleśny J. Spring rye as a source of biomass and carbon in the soil. *International Agrophysics*, 38, 243-255 (2024).

[2] Keskkonnaagentuur |[Keskkonnaagentuur](#) | ILM 17.03.2025

## Influence of long-term nutrient deficiency on pollen and anther morphological traits in rye

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Pollination performance relies on the proper development of anthers and the production of viable pollen, ensuring reproduction and allelic reshuffling. While abiotic factors such as temperature are known to impair this development, the influence of soil nutrient availability on male reproductive traits is less well understood and the underlying genetic mechanisms remain unclear (Young and Stanton 1990; Lau and Stephenson 1994).

Our study aims to investigate pollen and anther morphology in two rye populations – a population variety and a diversity panel – both grown under control and nutrient deficiency caused by over 140 years of monoculture. The goal is to understand the genetic architecture underlying pollen and anther morphology in responses to nutrient stress.

Pollen morphology was quantified using high-throughput imaging flow cytometry, pollen count per anther was measured via impedance cytometry, and anther length was assessed using light microscopy. Our results show that both anther length and pollen count per anther are significantly reduced under nutrient stress, while pollen size remains unaffected. Anther length and pollen count were positively correlated in both treatments. Under control conditions, a negative correlation between pollen size and count suggested a trade-off between size and number, which disappeared under nutrient stress, indicating a shift in resource allocation strategy to maintain pollen size. Genome-wide association scans for pollen and anther length revealed distinct quantitative trait loci (QTL) sets for each trait under both nutrient conditions, highlighting genotype-by-environment interactions.

Our study extends our understanding of the genetic basis of pollen and anther traits in response to nutrient stress and provides insights into the mechanisms that make rye a great cross-pollinator.

1. Lau T.-C., Stephenson A. Effects of Soil Phosphorus on Pollen Production, Pollen Size, Pollen Phosphorus Content, and the Ability to Sire Seeds in Cucurbita Pepo (Cucurbitaceae). *Sexual Plant Reproduction*, 7, 215-220, (1994).
2. Young H. J., Stanton M. L. Influence of Environmental Quality on Pollen Competitive Ability in Wild Radish. *Science*, 248, 1631–1633, (1990).

## **RYE2050: A Vision for Evolutionary Rye Breeding**

**Bernd Hackauf**

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Random-mating populations accelerate adaptation by modulating meiotic recombination, the key driver of genetic diversity. In rye, these populations act as reservoirs of untapped diversity, offering climate-responsive traits for both direct cultivation and hybrid breeding [1]. Long-term *in situ* cultivation has led to directional selection for stable grain production under increasingly variable environmental conditions.

The unpredictability of climate change necessitates breeding strategies that enhance resilience while reducing environmental impact. Evolutionary Rye Breeding (ERB) provides a dynamic, decentralized approach by maintaining high genetic diversity, enabling continuous adaptation to stressors such as drought, disease, and soil degradation. Unlike hybrid breeding, which often restricts genetic variation, ERB stabilizes yields, reduces input dependence, and enhances long-term sustainability. Multi-environment trials across European and Canadian rye-growing regions demonstrate the potential of ERB in terms of agronomic and grain quality parameters.

As the yield gap between hybrid and population rye remains smaller than in maize, ERB presents a viable alternative for breeding resilient cultivars. Overcoming current challenges in selecting against the undesired recessive wild-type allele of the GA-sensitive dwarfing gene *Ddw1* in seed multiplication will enhance genetic gain for plant height and lodging resistance in open-pollinated populations. Semi-dwarf random-mating rye populations further expand the genetic reservoir for climate adaptation. By re-activating the EUCARPIA rye phenotyping platform for ERB, we can harness evolutionary forces to strengthen agricultural resilience in the face of climate volatility.

[1] Hackauf B., Siekmann D., Fromme F.J. Improving Yield and Yield Stability in Winter Rye by Hybrid Breeding. *Plants*. 2022; 11(19):2666.

## **Pseudonymized phenotypic and genotypic data from two stages of rye (*Secale cereale*) hybrid breeding program as implemented during 2018 to 2021 at KWS SAAT SE & Co. KGaA**

KWS SAAT SE & Co. KGaA

In the field of plant breeding, publicly available datasets representing commercial breeding programs are scarce. Here we provide an overview of two published datasets from the rye breeding program at KWS SAAT SE & Co. KGaA that were made available as a resource for public research.

The datasets correspond to a breeding program for rye hybrids which involves crossing and development of parental inbred lines and multistage general combining ability (GCA) testing using testers from opposite heterotic pools. Rye hybrids in this program are derived from a cross between a CMS (cytoplasmic male sterile) intra-pool hybrid as seed parent and a restorer synthetic as pollinator parent. The datasets presented here correspond to a subset of genotypes of the phenotypic records from stages 1 and 2 of GCA tests implemented as part of the multistage hybrid rye program. The dataset 1 contains design effect corrected BLUE (best linear unbiased estimates) values for plant height and grain dry matter yield for each physical trial. The BLUE values have not been adjusted for the trial effect. The genotypes tested belong to three consecutive cycles of GCA testing in the years 2018-2019, 2019-2020 and 2020-2021. In each cycle, selected genotypes from GCA1 were passed on to the next year of testing (GCA2). Dataset 2 contains raw phenotypic records from a single year of testing i.e., including design effects for the same traits. The observations were made on hybrids in both datasets. There is no overlap between the two datasets for the hybrids evaluated and hence both are presented separately along with their respective genotype data.

We encourage scientists to download, analyze and publish results leveraging these datasets to collectively gain insights into methods of quantitative genetics and breeding methodology as well as their implications for commercial breeding programs.

## Powdery Mildew Resistance in Rye

**Christina Rønn Ingvardsen<sup>1</sup>, Jihad Orabi<sup>2</sup>, Khalid Mahmood<sup>2</sup>, Nana Vagndorf<sup>2</sup>, Marius Mohlfeld<sup>3</sup>, Jasmin Vettel<sup>3</sup>, Pernille Merete Sarup<sup>2</sup>, Ahmed Jahoor<sup>2,3</sup>, Henrik Brinch-Pedersen<sup>1</sup>**

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Rye is predominantly grown for food. The Danish cultivation area with rye is increasing rapidly and rye is becoming more and more economically important. However, the combination of powdery mildew (*Blumeria graminis*) favorable conditions and increased rye cultivation calls for a non-pesticide solution to the lack of mildew resistance in rye. We aim to solve this problem by introducing *mlo* resistance into rye. Mildew locus O (*Mlo*) is a plant-specific gene family, where specific members of the gene family act as powdery mildew susceptibility factors. A proper functional *Mlo* protein is required for successful penetration of the powdery mildew pathogen into the epidermal cell. Mutations in the gene result in an unstable or non-functional protein resulting in powdery mildew resistance. *mlo* resistance is well known from barley but has also been developed in other plant species, including wheat and soybean. As rye is an outbreeder and modern rye varieties are made via hybrid breeding, recessive *mlo* alleles need to be introduced in both restorer and non-restorer lines. To obtain *mlo*-resistant plants of both lines, we plan to use two strategies: developing and screening TILLING populations for both the restorer and the non-restorer populations, as well as mutating the *Mlo* gene using CRISPR/Cas9. A TILLING protocol for rye hybrids was optimized using three mutagenic treatments with EMS and NaN<sub>3</sub>, employing approximately 6,250 seeds of both restorer and non-restorer genotypes in each treatment. The treated seeds demonstrated good germination rates when sown in field. To develop the M2 population, 5,000 seeds from each component will be bagged to screen in future. To obtain exact sequence data for design of sgRNA for the CRISPR/Cas9, we sequenced the *Mlo* gene from 24 lines.

The presence of *mlo* powdery mildew resistance in rye has the potential to play a significant role in reducing dependency on pesticides and thus lead to an increase in sustainability and biodiversity.

## Implementation of a system for identifying the purity of rye inbred lines in the rye breeding program of Danko Plant Breeding

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The idea of breeding hybrid rye is to increase the fertility of the obtained varieties compared to traditional population varieties. For this purpose, the phenomenon of heterosis is used, which involves crossing homozygous forms at loci that are key to fertility. Hybrid varieties are three-component hybrids, created by crossing a male sterile line with a complementary one, and then the fertility of the resulting sterile hybrid (single) is restored with a restorer line. In the breeding program, as well as in the production of the hybrid itself, it is very important to maintain the purity of male sterile lines and sterile single hybrids, especially in terms of the presence of fertile plants. As part of the project entitled: " New plant variety breeding - modern varieties of selected species of cereals and pea, based on innovative biotechnological methods " implemented under the Intelligent Development Operational Program 2014-2020, Professor Stojalowski from ZUT in Szczecin was commissioned to develop a system for identifying the purity of inbred lines of hybrid rye , by detecting the presence of other genotypes in them. A methodology was obtained describing the calculated optimal batch size of the tested material, the recommended DNA isolation method, the method of creating a collective sample, a set of 10 SSR markers with given sequences, the optimized composition of the reaction mixture and the PCR reaction conditions. The system was tested on a batch of plants of three male sterile inbred rye lines and three male sterile singles. Analog lines with the N cytoplasm variant and genotypes tested in parallel were used as the source of contamination. It was confirmed that the system could be useful for detecting foreign genotypes in hybrid components.

Project under the title „New plant variety breeding - modern varieties of selected species of cereals and pea, based on innovative biotechnological methods” co-financed by the European Regional Development Fund under the Smart Growth Operational Programme 2014-2020, Action 1.1 R&D projects of enterprises, Agreement No.POIR.01.01.01.-00-1363 / 15, years of research 2016-2022

## DARtseq markers associated with restoration of male fertility in the F<sub>2</sub> population of unregistered Polish hybrid cultivar RPD1273

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The hybrid RPD1273 is an unregistered Polish cultivar based on the Pampa sterility-inducing cytoplasm. A single plant of RPD1273 was self-pollinated and obtained 179 individuals of F<sub>2</sub> population were phenotyped in the field experimental station of West Pomeranian University of Technology in Szczecin. Male fertility of plants was assessed by two methods: visual observations according to the Geiger and Morgenstern [1] 9-step scale and by frequency of seeds setting in isolated ears. Statistical analysis of phenotypic results excluded monogenic inheritance of studied trait. As the most probable hypothesis has been considered model with activity of 3 nuclear genes. From the entire F<sub>2</sub> population, 94 individuals were randomly selected and genotyped using the DARtseq platform (provided by Diversity Arrays Technology Pty, Bruce, Australia). The analysis resulted in a total of 63306 marker data, of which 5410 segregated according to the monogenic inheritance model. These markers were used for statistical analysis to indicate their association with phenotypic data. For this purpose, the Kruskal-Wallis test was used. A total 281 markers revealing statistical association with male fertility of plants have been found. They were distributed on various chromosomes (or were not assigned to the chromosome), but it has been noticeable that two numerous groups of markers were located on chromosomes 1R and 6R. These results are consistent with the localization of restorer genes for rye with Pampa cytoplasm shown by Miedaner et al. [2].

**Acknowledgments:** This research was supported financially by Polish Ministry of Agriculture and Rural Development under the Programme of Basic Research for Biological Progress in Plant Production (task 10)

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## Enhancing allele-specific PCR-based genotyping with a novel *in silico* designed enzyme: from breeding to seed quality control

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Allele-specific PCR assays are a cornerstone technology for SNP genotyping in agriculture, widely used for plant breeding, seed quality control, trait verification, and germplasm management. Its flexibility, cost-efficiency, and ability to handle large sample volumes make it ideal for modern, high-throughput genotyping workflows.

We introduce a new PCR mix for allele-specific, high-throughput genotyping, powered by a novel *in silico* designed DNA polymerase. This genetically engineered enzyme offers enhanced allele specificity, superior mismatch discrimination, and optimized kinetics for rapid thermal cycling — all critical for scalable agricultural genotyping.

Integrated into a streamlined cassette-based platform, the system enables fast and automated sample processing with minimal hands-on time. The product is fully compatible with existing KASP and PACE assays, and usable as a direct replacement for the associated PCR mixes. Benchmarking across key crops such as maize, oat, and tomato demonstrated high call rate accuracy, reduced nonspecific amplification, and excellent performance with variable DNA quality — including crude extracts and aged samples.

# SESSION III

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## **RYE DISEASE RESISTANCE AND STRESS TOLERANCE**

## Breeding for fungal disease resistances in small-grain cereals

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The aim of all breeding is to achieve genetic improvement in important traits, of which disease resistance is one of the most important. Resistance to powdery mildew in wheat and hybrid rye, leaf rust and *Rhynchosporium* leaf blotch in hybrid rye has shown a high genetic gain in a long-term study, whereas it is lower for *Septoria nodorum* blotch and absent for leaf rust, *Septoria tritici* blotch, yellow rust and Fusarium head blight (FHB) in wheat [1,2]. The durability of disease resistances is mainly determined by the mode of inheritance (monogenic vs. oligo-/polygenic) and the population dynamics of the fungal pathogens. In Europe, inheritance is mainly based on monogenic resistances in wheat leaf and stem rust, a mixture of monogenic and quantitative resistances in wheat yellow rust and pure quantitative resistances in FHB in wheat, triticale, and rye. Breeding progress in the latter is hampered by some agromorphological traits such as anther extrusion in wheat and plant height, especially the use of the dwarfing genes *Rht-B1*, *Rht-D1* (wheat) or *Ddw1* (triticale, rye) [3,4]. FHB resistance, although difficult to achieve, is one of the most durable resistances available. Leaf rust resistance, on the other hand, has the highest ageing effects of all cereals [1], *i.e.*, resistance is significantly reduced over the life of a variety due to adaptation of the highly dynamic leaf rust populations by frequent sexual recombination. Older concepts such as varietal mixtures or segregating varieties also improve the durability of some pathosystems. Genomic selection (GS) is superior to marker-assisted selection in quantitative traits and allows simultaneous selection for other traits of interest. In the future, genome editing could provide a whole range of new resistance alleles that could, for the first time, outpace the high reproduction rate of pathogen populations.

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## Rare missense mutations in the C terminal and NB-ARC domain of pm3 like disease resistance protein confer powdery mildew disease resistance in rye

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Powdery mildew ranks among the most destructive diseases globally, leading to significant grain yield losses and reduced cereal crop quality. The disease-resistance genes on the rye 1R chromosomal arm have played a vital role in controlling powdery mildew in wheat. This study investigates powdery mildew resistance in the elite restorer germplasm of Nordic Seed hybrid rye. A biparental mapping population was developed using inbred restorer genotypes with contrasting phenotypes for powdery mildew resistance. Genotyping was performed using a 30K SNP chip, and phenotyping was conducted on 184 individuals in the F2 generation. Markers associated with resistance were identified through genome-wide association studies (GWAS).

With the help of recent advancements in rye genomic resources, the SNP marker associated with powdery mildew resistance led us to the identification of "SECCE1Rv1G0001880" as the candidate gene which is annotated as NB-LRR like disease resistance protein.. Within this gene, missense mutations were detected in the CC and NB-ARC domains, likely conferring resistance to powdery mildew. Validation was achieved using PCR Allele Competitive Extension (PACE) markers targeting these SNPs in the biparental mapping population. The identified SNPs—causing E96K, F114L, and Q301K substitutions—were present in resistant individuals but absent in susceptible ones. To our knowledge, this is the first report of these specific mutations within a pm3-like disease resistance gene as contributors to powdery mildew resistance in rye.

**Keywords:** Hybrid breeding, SNP array, *Blumeria graminis* f. sp. *secalis*, nucleotide-binding leucine-rich repeat (NLR), NB-ARC domain, phylogenetic analysis, *NB-LLR* disease resistance protein

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## Comparative transcriptomics of rye (*Secale cereale* L.) inbred lines revealed candidate genes involved in phosphorus deficiency

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Phosphorus is one of the most limiting factors for plant development, and its low availability in agricultural lands negatively affects crop production[1]. Rye's resilience to low-nutrient stresses makes this grass a good model for studying mechanisms of tolerance to phosphorus deficiency. Our research aims to identify loci contributing to phosphorus-deficiency resilience through comparative transcriptomics of rye genotypes with contrasting response to phosphorus-deficient conditions.

We screened various rye inbred lines under phosphate-deficient conditions in a hydroponic-growing system. We measured fresh and dry weight and inorganic phosphorus content in leaves and roots. In our screening, we observed that the rye L9 and K3 inbred lines exhibited contrasting inorganic phosphate accumulation in leaves; the L9 line contains higher phosphate than the K3 line under phosphate deficiency. We performed a comparative transcriptomic analysis of these two inbred lines. The Principal Component Analysis (PCA) of the RNA-seq revealed that the genotype explains 79% and 81% of the variation between samples in leaves and roots, respectively. Also, PCA confirmed that the samples of the same genotype and condition group together, indicating a successful experimental stage. We analyzed the transcriptome data considering the genotype-environment (GxE) interaction effect (IE). We identified that phosphorus deficiency affects the gene expression of 456 and 3944 genes in roots and leaves across both phenotypes differently, respectively. In leaves, we identified a few DEGs involved in phospholipid metabolisms, which might be involved in membrane remodeling, while in roots, we identified several transporters that might play a role in the translocation of inorganic phosphorus. Our results will allow us to select candidate genes conferring high-phosphate content in leaves during low-phosphate regimes.

**Acknowledgments:** This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

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## Impact of the GA-sensitive dwarfing gene *Ddw1* on the performance of rye under drought

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The outcrossing cereal rye (*Secale cereale* L.) serves as a proven model of resilience. Thriving in poor soils and harsh climates where other cereals falter, rye's adaptability over millennia—flourishing without intervention—demonstrates its exceptional capacity to endure and produce under challenging conditions. Hybrid rye breeding has enhanced grain production on finite arable land without increasing water and fertilizer use. However, no significant genetic gain has been achieved in plant height and lodging resistance of rye over the past 30 years [1].

To address this, we have improved target-specific selection efficiency for the introgression of the GA-sensitive dwarfing gene *Ddw1* into the seed-parent pool [2]. Here, we evaluate the effects of *Ddw1* on rye performance under drought. For this purpose we present a phenotyping platform as effective experimental environment for drought stress. Comprehensive precision phenotyping of semi-dwarf and standard hybrids under controlled drought stress revealed pronounced genetic variation in agronomic and quality traits. We identified key traits influencing rye performance under drought, contributing to our understanding of whether plant height in rye is a crop-specific trait.

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## Viral diseases transmitted by insects in rye

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In recent years, there has been a growing problem of insect transmitted virus infection in rye in the Lower Silesia region in Poland. Plant samples showing disease symptoms were analyzed using ELISA tests, which detected two main pathogenic agents: barley yellow dwarf virus (BYDV) & wheat dwarf virus (WDV). BYDV is one of the most dangerous and widely distributed viral diseases affecting cereals. BYDV is caused by several virus species that are transmitted by aphids, leading to significant yield losses, especially in winter barley with an increasing number of cereals affected year on year. Characteristic disease symptoms of BYDV include growth inhibition and leaf discoloration, which in rye appears as a rusty hue. Affected plants are shorter than healthy ones and are characterized by a changed habit and stiff leaves. In severe BYDV cases, the plants die. WDV affects all cereal species, leading to significant growth inhibition and eventual death of the plants. WDV is transmitted by leafhoppers. WDV infections are primarily seen at field edges and along pathways, but there has been a recent trend of entire fields being affected.

Compared to other cereals, rye as a species was considered more resistant to insect transmitted viral diseases. Unfortunately, in recent years it has become apparent that rye is increasingly susceptible to infection. Such susceptibility is particularly noticeable in breeding fields, where single plant sowing is common, whereby the density of plants per unit area is low. Currently, the primary methods for preventing BYDV and WDV infection involves delaying the sowing of winter crops and applying intensive insecticide protection. However, the effectiveness of these strategies is limited due to the increasing duration of the vector insect infestation, and the restricted availability of insecticides for their control in rye.

As global warming progresses, pressure from insect borne viral diseases in rye is expected to intensify. In this situation, the best solution is the development of rye varieties less susceptible, or more resistant, to viral diseases. This goal appears attainable due to the high genetic variability for resistance to BYDV and WDV observed in rye breeding material.

## The Daylength Dilemma: Photoperiod Sensitivity in Rye

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Flowering time is a key adaptive trait, enabling correct synchronization of plants to their environment. Many cereals are long day plants, flowering as daylength increases. In wheat & barley genetic variation in *Ppd-1* explains >60% flowering time variation. Whilst rye encodes a *Ppd-1* ortholog (*Ppd-R1*) its precise role is unknown, with other potentially more significant flowering time loci on chromosomes 5R, 6R & 7R reported<sup>1,2</sup>.

We collected and screened rye germplasm of diverse geographic origin to identify variation in the day-length requirement for flowering. When grown under short day conditions we identified multiple photoperiod insensitive genotypes, with variation in vernalization requirement also observed. The majority, 75%, of the photoperiod insensitive types identified were of Mediterranean origin.

Development and assessment of a mapping population segregating for photoperiod sensitivity facilitated QTL discovery. Here, we identified flowering time QTL, both concordant and different to those already reported<sup>1,2</sup>. Of these QTL, many encompass orthologs of flowering time gene candidates known from the Triticeae and wider Poaceae relatives. However, compared to wheat and barley, *Ppd-1* may play a lesser role in day length sensitivity of rye, whereby QTL encompassing orthologs of 'minor' flowering time genes appear to take centre stage.

Together, these results may hint at a broader genetic base of flowering time in rye compared to its Triticeae relatives. Conversely, such divergence may reflect the relatively shorter history of rye domestication, lower selection intensity, and the comparative geographic restriction of cultivation. Just as photoperiod insensitivity was selected during wheat and barley domestication, understanding how rye responds to day length would facilitate adaptation to lower latitudes and rye cropping area expansion.

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## Winter rye variety 'Kaupo' response to temperature and precipitation: a comparative study in Priekuļi and Viļāni, Latvia

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This study looks at how different levels of temperature and rainfall affect the performance of the local winter rye variety 'Kaupo' in two locations in Latvia from 2012 to 2023. Priekuļi and Viļāni have experienced increased average annual temperatures and altered precipitation patterns since 2012. Phenological phases—sowing time, heading, and maturity—and quality traits—winter hardiness, lodging resistance, plant height, yield, protein content, and falling number—were analysed. Despite an increase in the average air temperature in September and a later end of the vegetation period, the sowing date of rye has remained almost constant—the first or second decade of September. There is a significant relationship between winter hardiness, lodging resistance, plant height, and yield. The highest yields in both locations were achieved in years when winter hardiness reached 90-100% and weather conditions were optimal for growth and yield formation. In 2013 and 2023, winter had high precipitation; growth resumption was late, and there were prolonged periods of drought in the spring. As a result, the rate of winter hardiness was low, plants did not form a dense canopy, the stems were short, and yields were low. The results also demonstrate a significant impact of extreme weather conditions on yields. Prolonged and heavy rainfall in Viļāni in 2017 resulted in a very late harvest (August 31) and a low falling number (73 sec). In Priekuļi in 2017, the rye was harvested before the period of intense precipitation began, so the falling number remained high (286 sec). In 2020, hail broke off 80% of the plants during flowering. As a result, the yield was 2.5 times lower than in years when conditions were favourable for growth and development throughout the growing period. Data analysis was limited by several missing values in the data from both study sites. The results of this study indicate that weather conditions can be unpredictable, and these results can contribute to a better understanding of the breeding potential for developing climate-resilient varieties.

## Proteome profiling of rye (*Secale cereale* L.) L9 inbred line seedlings under phosphorus deficiency

**Brian Wakimwayi Koboyi<sup>1</sup>, David Chan-Rodriguez<sup>1</sup>, Sirine Werghi<sup>1</sup>, Hanna Bolibok-Brągoszewska<sup>1\*</sup>**

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Phosphorus (P), an essential macronutrient, is pivotal in plant growth and development since its deficiency directly impacts agricultural productivity. Therefore, unravelling the intricate mechanisms through which certain crops tolerate phosphorus deficiency could facilitate the improvement of susceptible ones through breeding to increase crop yield.

We aim to study the mechanism of phosphorus tolerance in the rye (*Secale cereale* L.) shoots and roots.

We standardised a phenol-based total protein extraction method after comparing the protein yield, pellet dissolution, extraction duration and band quality on 10% SDS-PAGE gels between Method 1 (SDS-containing extraction buffer + trichloroacetic acid clean-up step) and Method 2 (SDS-containing extraction buffer + phenol clean-up step). Additionally a chloroform-methanol precipitation step was included to obtain pure protein suitable for liquid chromatography/tandem mass spectrometry (LCMS/MS) by eliminating Sodium Dodecyl Sulphate (SDS) that interferes with downstream mass spectrometry analyses (1,2).

Protein was extracted from shoots and roots of 21-day-old L9 inbred line rye (*Secale cereale* L.) seedlings grown in a hydroponic system of 0.2 mM KH<sub>2</sub>PO<sub>4</sub> in control (+P) conditions and 0.01mM KH<sub>2</sub>PO<sub>4</sub> (with additional 0.19mM KCl in phosphorus-deficient conditions (-P)). The isolated proteins were analysed via LCMS/MS with Tandem Mass Tag labelling (TMT).

With reference to the *Secale cereale* database, 157 proteins were identified. Among them, 114 proteins were differentially expressed, 54 upregulated, and 60 downregulated. The Principal Component Analysis clustered the identified proteins into four groups, reflecting tissue type (shoots or roots) and growth media (control or phosphorus-deficient media) as the differentiating factors.

**Acknowledgements:** This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

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## SNP-Based Linkage Mapping in Rye F<sub>2</sub>: A Step Toward Uncovering Brown Rust Resistance

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In this study, we constructed a dense linkage map for an F<sub>2</sub> rye (*Secale cereale*) population using an Illumina Infinium microarray approach that primarily employed the Rye 5K SNP set (Haseneyer et al., 2011), alongside selected markers from the Rye 600K array and candidate gene loci associated with disease response. The F<sub>2</sub> population originated from a cross between a brown rust-resistant line and a susceptible line, creating a valuable resource for dissecting quantitative trait loci (QTL) linked to disease tolerance. Genomic DNA was isolated from each F<sub>2</sub> plant using a commercial kit, ensuring uniform quality and concentration, which facilitated accurate hybridization on the microarrays. After scanning, raw signal intensities were converted into SNP genotype calls, resulting in a comprehensive dataset of polymorphisms segregating in the F<sub>2</sub> lines. All markers were rigorously tested for adherence to the expected 1:2:1 segregation ratio using a chi-square test, and only those meeting strict significance criteria were retained for linkage analysis. This filtered marker set was imported into R and processed with the ASMap package (Taylor and Butler, 2017), where pairwise recombination frequencies and log of odds (LOD) scores were used to assign markers to seven linkage groups corresponding to chromosomes 1R through 7R (Rabanus-Wallace et al., 2021). Ultimately, 962 unique loci were identified, yielding a total map length of 4469 cM and an average inter-marker spacing of 3 to 6.4 cM. While this coverage is generally robust, one notable gap of over 80 cM on chromosome 5R may reflect localized genomic complexity or lower recombination rates. Apart from this region, the map exhibits minimal segregation distortion and aligns well with earlier high-density linkage studies in rye (Milczarski et al., 2016), underscoring both its reliability and its potential for further genetic analyses. The resulting linkage map not only provides an effective framework for identifying QTLs related to brown rust resistance but also offers a valuable tool for dissecting other agronomically important traits. By pinpointing genomic regions underlying disease tolerance, this study sets the stage for informed breeding strategies that aim to improve rye cultivars, aligning with broader efforts to enhance cereal crop resilience and yield (Zhou et al., 2016).

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## Epigenetic regulation of phosphorus deficiency response in rye (*Secale cereale* L.): A DNA methylation perspective

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Phosphorus (P) deficiency triggers various adaptive responses in plants, including changes in DNA methylation, which often correlate with altered gene expression [1]. Rye (*Secale cereale* L.), a stress-tolerant cereal related to wheat and barley, exhibits enhanced P deficiency tolerance. DNA methylation and other epigenetic mechanisms like histone modifications and small RNAs play a key role in regulating gene expression in response to environmental stress. The rye genome, rich in heterochromatin and transposable elements, suggests that DNA methylation may be particularly important for its genetic plasticity and adaptability to environmental changes[2].

The aim of this study is to understand the molecular mechanisms underlying rye's tolerance to P deficiency by characterizing changes in DNA methylation. Specifically, we aim to investigate methylome regulation under nutrient stress and provide a basis for comparative analyses of DNA methylation dynamics upon P starvation among rye. Based on a P deficiency response screen in hydroponic conditions, two inbred lines of rye, L9 and K3, exhibiting a contrasting response, were selected. DNA was isolated from the roots and shoots of 14- and 21-day-old plants grown under phosphorus-deficient and control conditions. Methylation status was examined via Methyl Sensitive DArT sequencing (DArTseqMethyl) at Diversity Arrays Technology (Bruce, Australia). Methylation conversion was performed using the NEBNext Enzymatic Methyl-seq Conversion Module.

Our findings highlight the role of DNA methylation in rye's response to phosphorus deficiency. The stability of CpG and CHG methylation suggests their importance in genome integrity, while elevated CHH methylation may indicate epigenetic regulation of transposable elements in rye. Variations in phosphorus transporters (*ScPht*) gene methylation patterns suggest adaptive regulatory mechanisms influenced by stress, chromatin accessibility, and environmental factors, underscoring the dynamic role of DNA methylation in nutrient stress adaptation.

**Acknowledgments:** This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

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## Mature embryo cultures as a tool for testing rye's response to abiotic stresses and nutrient deficiencies

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Nitrogen (N) and potassium (K) are elements of particular importance; their deficiency significantly limits the adaptive capacity of plants to unfavorable environmental conditions. Genotypes that tolerate stress at the seedling stage may retain this trait at the mature plant stage though not always. If, at the seedling stage, they effectively activate adaptive mechanisms, such as the expression of specific genes or morphological adjustments of the root system to stress conditions, the plant may maintain its tolerance in later developmental stages. The primary goal of our research is to develop a test for the effective selection of, among others, rye genotypes tolerant to N, K, and combined N and K deficiencies at the seedling stage using mature embryo cultures. This goal is pursued through specific objectives, which include: (i) describing the response of rye genotypes to N, K, and combined N and K deficiencies in the medium; (ii) refining the methodology for inducing a controlled gradient of N or K ion concentrations in the medium, allowing for real-time induction and subsequent measurement of the plant's response to changing stress conditions this methodology is being developed based on our previous results (Smolik and Smolik, 2024); and (iii) shortly, investigating and comparing the transcriptomic responses (Xu et al., 2011) of rye genotypes classified as tolerant and susceptible to nutrient stress. The research material consists of selected rye inbred lines, RILs ( $F_{>15}$ ), as well as population and hybrid cultivars (Danko Hodowla Roślin Sp. z o.o., Poland). Phenotyping is conducted by modifying methodological elements previously described by Rzepka-Plevneš et al. (1997) and Smolik (2013a, b). At this stage of the study, mature embryos are cultured on media with varying N content. In addition to seedling biometrics, we also examine the biometrics of grains and embryos and the agronomic traits of mature plants. The results are analyzed using, among other methods, RGCCA. We have demonstrated that the studied rye genotypes exhibit varying, often extreme, responses to stress induced by nitrogen deficiency.

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### SESSION III: RYE DISEASE RESISTANCE AND STRESS TOLERANCE

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## Development of Organic High-Quality Rye and Lupin for Human Consumption (RUPIN)

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The "Development of Organic High-Quality Rye and Lupin for Human Consumption (RUPIN)" is a four-year research project funded by International Centre for Research in Organic Food System (ICROFS) of GUDP, Denmark. The project is led by Nordic Seed in collaboration with Teknologisk Institut, University of Copenhagen, and Aarhus University. The project aims to improve the quality and yield of rye and lupin cultivars specifically suited for organic farming. Rye is the central focus of the project, given its significance in the organic market and its nutritional benefits. With its high fiber content, rye is ideal for whole grain products, yet current methods for assessing its baking quality are inadequate. Therefore, this project aims to improve the rye quality for human consumption and develop cultivars suitable for organic farming.

In this project, we already started the production of 194 hybrids and 6 population that will be sown in Autumn, 2025. This project is structured into five work packages (WPs). WP1 emphasizes field trials of rye hybrids and populations under organic and semi-organic conditions. The field trials will be conducted at three locations - Nienstädt (semi-organic) and two Danish sites (organic) - over two years. Along with quality related traits, we will also assess several other traits that include agronomic (lodging, straw strength, plant height), disease resistance (Scald, Brown Rust, Stem Rust, Mildew), and yield. Seeds from trials will be pooled per replicate for comprehensive quality analysis in WP2. This work package will focus on rye quality, evaluating baking-related traits such as falling number, amylography, starch composition, protein, fiber, and water absorption capacity. WP3 uses the rye quality data to build predictive models for baking quality selection using genomic prediction.

This integrated, interdisciplinary and rye-focused initiative will provide organic farmers with superior rye varieties that meet quality standards for food production, expand sustainable cultivation on marginal lands, and support the increasing consumer demand for organic and whole grain products.

**Acknowledgement:** Funds allocation for this project by ICROFS, GUDP Denmark.

# SESSION IV

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**RYE FOR FOOD, FEED,  
FUEL AND FUN**

## Comin' thro' the rye

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Compared to wheat and barley, the history of cultivated rye (*Secale cereale*) as cereal crop began not before the European Bronze Age. Nonetheless, rye is deeply associated with civilization, food supply and cultural heritage in Central and Eastern Europe, and the Baltic Sea region [1]. Despite regional and EU wide efforts to promote traditional rye cultivation and products, e.g., the Rye Route in Estonia or the authorised EU health claim for rye fibre [2], the acreage of rye declined dramatically in the last half century (e.g., 70% in Germany and Estonia and 80-85% in Austria and Poland, respectively) [3]. Indeed, the unique characteristics and chemical composition of the rye grain [4], in particular the high level of soluble non-starch polysaccharides, are not only health beneficial but also hamper the large-scale use of rye for food, feed and fuel. Moreover, maximum levels for ergot sclerotia/alkaloids in unprocessed grains and milling products are further reduced by EU policy in the near future [5]. Hence, challenges for rye production and marketing are assumed to rather increase than decrease. Especially, small-scale production areas with lower grain yields may be given up and lead to a further decline of rye acreage. The limitations of rye with respect to food, feed and fuel processing will be outlined as well as possibilities to develop new niche markets.

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## Breeding cereal rye for cover crop and forage in the United States

**Lisa Kissing Kucek, Virginia Moore, Chris Reberg-Horton, Ramon Leon, Nancy Ehlke, Steven Mirsky, Shawn Conley, Kristin Simons, Travis Witt, Dustin Herb, Kerry Clark, Marcelo Ayres Carvalho, Rodrigo Werle, Marta Kohmann**

Cereal rye acreage in the United States has rapidly grown over the last 15 years, now rivaling major crops like spring wheat and cotton. To support this burgeoning crop, the Cover Crop Breeding Network has created a nationwide selection and variety testing program for cereal rye. As a first step in the breeding process, Cornell University conducted a cereal rye survey to clarify grower and seed industry priorities for selection. Breeding nurseries in five different states (MD, MN, NC, NY, and WI) are selecting genotypes tailored to the diverse uses of cereal rye identified in the survey. Targets include biomass, late planting, establishment during drought, forage nutritive value, seed yield, and divergent selection for low and high allelopathy and/or early and late flowering. Many of these traits are also being evaluated in a nationwide coordinated rye variety trial for cover crop use across eight states (MD, MO, NC, ND, NY, OK, OR, and WI). To further understand how rye genotypes differentially perform among environments and cropping systems, the USDA-ARS is compiling historical data to create a global cover crop variety trialing database. Soybean growers have expressed strong interest in allelopathy to manage weeds, but growers of alfalfa and other allelopathy susceptible crops want to minimize damage to crops planted after rye. Trials with University of Wisconsin-Madison, USDA-ARS, and North Carolina State University are exploring the impact of rye genotype and management on allelopathy.

## **Partially perennial? Self-cover cropping cereal rye as a novel application for material produced from perennial grain breeding efforts**

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Cover cropping is a proven method for reducing nitrate leaching and soil erosion, however it entails additional costs and complexity for farmers. In Denmark, cover crop use following a winter cereal is mandatory as part of an overarching policy to reduce nitrate leaching. Therefore, practices that can offer the same nitrogen-retaining benefits as cover crops while eliminating the production challenges would be attractive to both policy makers and farmers. Wide hybridization between *Secale cereale* and a perennial ancestor *Secale strictum* and subsequent back-crossing done in Germany has produced cereal rye that produces moderate grain yields and is capable of post-harvest regrowth. Here, we propose a novel implementation of that material: a self-cover cropping cereal rye. In 2024 a trial was planted to compare the performance of three cereal rye-based systems: (1) an annual cereal rye, (2) an annual cereal rye followed by a fodder radish cover crop (*Raphanus sativus*), and (3) a self-cover cropping cereal rye (*Secale cereale* x *S. strictum*). These systems are being compared on their agronomic performance, fall nitrate leaching, and ability to provide additional ecosystem services such as enhanced fall weed seed predation. In this talk I present the conceptual idea of partially perennial grains, and preliminary results from the field experiment.

## Investigating the variability of free asparagine content in rye cultivars grown in Poland

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Acrylamide is a thermal processing contaminant found in foods processed mostly at temperatures above 120 °C formed from free asparagine in the presence of reducing sugars during the Maillard reaction (Govindaraju et al., 2024). Rye (*Secale cereale* L.) contains the highest level of free asparagine among small grain cereals, which may significantly contribute to the enhanced formation of acrylamide in rye bakery products. Agronomic and genetic research in raw materials is focused on getting grain with decreased asparagine and reducing sugars content (Knaur et al, 2024). Identifying rye cultivars with low asparagine content or optimizing agronomic conditions to produce raw material with decreased asparagine concentrations could reduce the levels of carcinogenic acrylamide formed in baked rye products (Postles et al., 2013).

The aim of our study was to investigate the formation of free asparagine content in grain of population and hybrid rye cultivars. 34 rye cultivars were grown over a period of two growing seasons in Poland. The integrated and intensive management intensity were used. Rye cultivars showed the wide range of asparagine content (370-1098 mg kg<sup>-1</sup>). Weather conditions during vegetation season significantly influenced free asparagine content (795 vs 544 mg kg<sup>-1</sup>, respectively). Hybrid rye cultivars were characterized by lower asparagine content than population cultivars in both crop years. Considering applied management intensity, higher asparagine content was found for rye cultivated in intensive system (721 vs 648 mg kg<sup>-1</sup>, respectively). Hybrid cv. 'SU Arvid' contained the highest level of asparagine (average 777 mg kg<sup>-1</sup>). This cultivar was also characterized by the highest differentiation between crop years and input management (35% RSD). The lowest asparagine content was found in hybrid cv. 'KWS Serafino' (539 mg kg<sup>-1</sup>) with also high RSD (32%).

This work is based upon the cooperation in COST Action 21149 ACRYRED.

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## Rye as a Valuable Cover Crop

**Liina Talgre<sup>1,2\*</sup>, Evelin Loit-Harro<sup>1</sup>, Viacheslav Eremeev<sup>1</sup>, Merili Toom<sup>1,2</sup>**

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<sup>2</sup>*Centre of Estonian Rural Research and Knowledge*

Rye (*Secale cereale*) is a cereal crop with a long history and widespread use in Estonia, cultivated for food, animal feed, and as a cover crop. Several studies conducted in Estonia confirm the suitability and benefits of rye as a cover crop. Its exceptional winter hardiness makes rye particularly valuable in this role, as most cover crops do not survive the winter in Estonian conditions.

The biomass production of rye depends on the weather conditions during the growing season, the time of sowing, and variety but it is generally lower than that of other cover crops. Trials conducted at the Estonian University of Life Sciences (EMÜ) and the Estonian Crop Research Institute have shown that rye biomass can vary significantly, ranging from 667 to 1486 kg ha<sup>-1</sup> at the end of the autumn growing period, potentially doubling by spring. Up to 37% of this biomass consists of roots. Compared to other cover crops, rye produces greater biomass even when sown late [1; 2; 3].

As a cover crop, rye effectively absorbs nitrogen (N), reducing nutrient leaching into deeper soil layers or water bodies. When incorporated into the soil in autumn, rye biomass returns 30–50 kg N ha<sup>-1</sup> to the soil, while in spring, this amount can reach approximately 80 kg N ha<sup>-1</sup> [1; 2]. By the time of incorporation (early May), the carbon-to-nitrogen ratio of rye biomass remains below 30, meaning it does not negatively affect the subsequent crop [3].

Long-term crop rotation trials at EMÜ have demonstrated that, despite its relatively low biomass, rye is highly effective in suppressing weeds. It establishes quickly and shades out weeds, creating an unfavorable environment for their development [4]. Additionally, rye produces chemical compounds that inhibit weed growth [5], helping to reduce the need for herbicide applications in subsequent crops.

Studies on cover crop mixtures conducted at EMÜ indicate that rye integrates well with other cover crops, contributing to increased biodiversity and improved soil nutrient balance [6]. Therefore, using rye as a cover crop is an environmentally friendly solution that supports the principles of sustainable agriculture.

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# SESSION V

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## **PAST & FUTURE OF RYE RESEARCH AND BREEDING**

## Genome editing in rye – lessons from other Triticeae cereals

### Jochen Kumlehn

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Plant Reproductive Biology

The establishment of Cas endonucleases as genome editing tools has expanded the possibilities of plant biotechnology in ways previously thought scarcely possible. We demonstrated the power of this technology by generating gene-specific mutants with agriculturally relevant traits in some cereals and other crops. For example, two-rowed barley was converted into the 6-rowed type via knockout of *VRS1*, and hulled barley was modified to form naked, edible grains through mutagenesis of *NUD1*. Genome editing also facilitates the advancement of other plant breeding technologies. By targeted knockout of *PLA1*, haploidy-inducing barley lines were developed that, when used as pollinators, lead to the generation of homozygous maternal recombinants. Utilizing such doubled haploid lines has proven to be extremely effective in crop improvement. Further, we used *cas9*- and (wheat-specific) guide RNA-transgenic maize lines to pollinate wheat. Relying on the phenomenon of uniparental genome elimination, this leads to the immediate generation of *cas9* and guide RNA transgene-free wheat lines that carry newly induced genome edits in the homozygous state. A key challenge of further technological advancement is to go beyond targeted mutagenesis by developing precise genome editing methods at an applicable level. For instance, base-editing Cas9 derivatives were used to precisely mimic specific, still functional barley *eIF4E* alleles that confer resistance to bymoviruses. In another example, the heavy metal transporter HMA3, that had been spontaneously disabled in the context of durum wheat domestication, was functionally restored via Cas9-triggered DNA cleavage followed by repair through homology-mediated end joining. The precise deletion of a mutative 17-bp duplication achieved in this approach is expected to result in a significant reduction of the accumulation of cadmium in the wheat grains. In rye, however, genetic engineering is still generally limited by the particularly tough challenge of establishing robust transformation methods. Preliminary progress has recently been achieved by co-expression of a growth-regulating factor that boosts adventitious shoot formation *in vitro*. This eventually provides the prospect of implementing options of genome editing for rye as well.

## Winter rye breeding progress-where do we stand?

**Henryk Bujak<sup>1,2</sup>, Tomasz Lenartowicz<sup>1</sup>, Anna Skrzypek<sup>1</sup>, Marcin Przystalski<sup>1</sup>**

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Progress in crop breeding can be defined as the sum of various elements of economic and utilitarian value contributed by newly developed varieties. The effect of creative breeding of crop varieties is the improvement of their development parameters, which are important both from the agrotechnical and utilitarian point of view. Among the most important of these are: increasing the level of fertility, improving the nutritional, fodder or technological value of the yield obtained, obtaining high resistance to biotic stresses (pathogens), physical stresses (deficits, harsh weather conditions) and others.

In the 1980s and 1990s, the area of rye cultivation in Poland was 2.5 million hectares, where mainly population varieties were grown. Today it is less than 650,000 ha. The first hybrid varieties were registered in Poland in 1998. In the following years, not many hybrid varieties were registered, mainly due to low resistance to diseases such as brown rust. In recent years, mainly hybrid varieties of rye have been registered. Many new varieties are registered every year in the CCA catalogue, while in Poland the national register contains 77 varieties of rye, of which 26 are hybrids, 23 population varieties for grain, 3 population varieties for green fodder and 23 components of hybrid varieties ([www.coboru.gov.pl](http://www.coboru.gov.pl)) (03.2025).

The aim of the present study was to analyse the genetic and non-genetic trends for yield and brown rust resistance in 63 Polish winter rye post-registration (PDO) trials. Based on the results of the last 25 years, we first compared the overall trend for yield in PDO trials with 66 on-farm yields.

It was shown that in the PDO trials conducted by COBORU between 2000 and 2024, the average rye yield was 71.2 dt/ha. In addition, both the yields received by farmers in those years and the average yield in the PDO trials increased by an average of 55 kg/ha.

Hybrid and population varieties have many characteristics in common, but they also sometimes differ significantly. The most important difference between hybrid and population varieties of rye is fertility. Research carried out by COBORU shows that the yields of the highest yielding hybrid varieties are usually about 17-19% (18% in 2024) higher than those of the highest yielding population varieties. Furthermore, based on 25 years of data, the yield of hybrid varieties was about 11 dt/ha higher than that of population varieties.

## History of KWS short hybrid rye: Applied product innovation and its value for farmers

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Improving standability, harvest index and yield are major goals in rye breeding. In wheat, the reduction of plant height through the introduction of dwarfing genes has proven successful in achieving these goals. While short-straw population rye varieties were already released in 1938 by F. von Lochow-Petkus GmbH, dwarfing genes have played almost no role in hybrid rye breeding so far. Although lodging has been improved in modern rye cultivars, it remains an important problem, only controllable by chemical growth regulator application. Moreover, the European green deal and the farm-to-fork strategy clearly promote for a move towards more sustainable agriculture, requiring a reduction in chemical applications. Consequently, we developed the first KWS short hybrid rye cultivar “KWS Erebor”, officially registered in 2025, containing a genetically controlled growth regulation to combine heterosis in yield with lodging resistance. Since little is known about the impact of dwarfing genes on yield performance and agronomy in rye, we conducted studies to investigate their effect on leaf diseases, behavior under high nitrogen soil conditions and the root system. Overall, short hybrid rye varieties offer new possibilities to farmers by combining several advantages: Their high yield and high lodging tolerance enables them to outcompete wheat and giving access to new growing areas, even under intensive cultivation conditions. The waiving of growth regulators, high drought stress tolerance, high uniformity of the vegetation and great water and nitrogen usage efficiency lead to a low carbon footprint, effecting economy and improving sustainability.

## New Perspectives for Rye: Facultative Hybrids

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Rye is a versatile cereal crop cultivated in temperate regions, known for its adaptability to adverse environments and exceptional tolerance to biotic and abiotic stresses [1]. However, rising global temperatures and shifting rainfall patterns through the year poses additional challenges for sowing and crop development. To address challenges from climate change and meet new agricultural demands, great efforts are needed in rye hybrid programs towards enhancing adaptation, phenology and plant architecture. Habit growth of annual cereals may be broadly classified into winter, facultative, and spring types, differing in seedling period due to different requirements for vernalization and photoperiod. Farmers in Central Europe and North America mostly cultivate the frost-tolerant winter type of hybrid rye, sown in autumn, which needs vernalization and long-day conditions in spring for induction of flowering. Spring genotypes, however, have lower requirements for vernalization [2], and thus can be sowed in late winter or early spring. In rye, the spring growth habit is dominant over the winter growth habit, therefore, crosses among winter x spring results in facultative types. Facultative rye hybrids combine winter hardiness with the ability of flowering without vernalization, enabling continuous growth at temperatures above 8°C. The integration of these traits into a hybrid promotes greater biomass accumulation, can lead to significant improvements in yield stability and drought tolerance through increased water retention on stems and better root development through autumn and spring. Their flexibility allows for both autumn and spring sowing, making them suitable for regions with mild winters. These hybrids offer agronomic advantages and open new market opportunities in diverse growing conditions, potentially new markets for rye and adding more options for farmers during sowing.

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## Rye research and breeding: looking back and forward

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Rye has been a crucial cereal crop, especially in Europe, due to its winter hardiness and ability to thrive in poor soil conditions. The natural genetic diversity of rye has been essential for its adaptation and improvement over centuries. Historically, rye was primarily used for bread making, but its use has diversified over time.

Molecular breeding has revolutionized rye breeding by enabling more precise and efficient development of new varieties. Molecular markers, such as Single Nucleotide Polymorphisms (SNPs), are used to identify and select desirable traits in rye. These markers help in tracking genes associated with disease resistance, yield, and quality traits.

Marker-Assisted Selection (MAS) uses molecular markers to select plants with beneficial traits during breeding programs, speeding up the process and increasing accuracy.

Genomic selection involves analyzing the entire genome of rye plants to predict their performance based on genetic data. This method allows breeders to select the best candidates for breeding without extensive field trials. By using genomic selection, breeders can enhance traits such as yield, drought resistance, and disease resistance more efficiently.

Advanced genome editing tools like CRISPR/Cas9 are being explored to make precise changes in the rye genome. This technology can be used to introduce or remove specific genes, improving traits such as disease resistance and environmental adaptability.

High-throughput phenotyping involves using automated systems to measure plant traits quickly and accurately. This data is then correlated with genetic information to identify the best breeding candidates.

Functional genomics studies help identify genes responsible for important traits in rye. Understanding these genes allows breeders to manipulate them for improved crop performance.

These molecular breeding techniques are transforming rye breeding, making it more efficient and targeted.

The talk will also include the history of EUCARPIA RYE and a personal view on rye research and the rye research community over the last 30 years.

### **Further reading:**

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