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BOOK OF ABSTRACTS

Ulrike Lohwasser & Andreas Börner
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Program

June 21, 2021

09:00-09:20	Andreas Börner, Andreas Graner Germany	Opening Remarks
	Session I	**Rye Genetic Resources and Diversity**
	Chair: Andreas Börner	
09:20-09:50	**Plenary Talk**	
	Jochen Reif Germany	Genebank2.0: Activation of the IPK winter wheat collection for breeding purposes - lessons learned and significance for pre-breeding in rye
09:50-10:10	Mona Schreiber Germany	Not all bread is baked in one oven & not all rye is of simple descent
10:10-10:30	Mira Ponomareva Russia	Application of genetic resources for rye breeding in Volga Region of Russian Federation
10:30-10:50	Steven Dreissig Germany	Using a 140-year-old rye monoculture field trial to investigate how genetic and environmental factors affect genetic recombination
10:50-11:10	Jianyong Chen Germany	Identification of factors that make the B chromosome of rye deviate from the Mendelian law of equal segregation
11:10-11:30		**Coffee Break**
	Session II	**Breeding Methods and Molecular Tools**
	Chair: Viktor Korzun	
11:30-12:00	**Plenary Talk**	
	Nils Stein Germany	Rye genome sequenced - what`s next?
12:00-12:20	Bernd Hackauf Germany	Bridging the genotype-phenotype gap for SMART breeding in rye
12:20-12:40	Andres Gordillo Germany	Genomic selection in rye hybrid breeding: from theory to practice
12:40-13:45		**Lunch Break**

13:45-14:05	Xiangyu Guo Germany	Genomic prediction of yield in hybrid rye
14:05-14:25	Anatolii Goncharenko Russia	Breeding of winter rye hybrids at Federal Research Center "Nemchinovka"
14:25-14:45	Stanislau Hardzei Belarus	Hybrid rye in Belarus: breeding results, cultivation
14:45-16:15	**Chair: Andreas Börner**	**Meet the Sponsors/Elevator Pitch I**
16:15-16:30		**Coffee Break**
	Chair: Bernd Hackauf	
16:30-17:00	**Plenary Talk**	
	Jochen Kumlehn Germany	Site-directed genetic engineering in cereals - Principles and applications
17:00-17:20	Diaa Eldin S. Daghma Germany	Establishment of adventitious shoot formation in vitro and a genetic engineering platform for rye
17:20-17:40	Khalid Mahmood Denmark	Genomic scan of male fertility restoration in Gülzow' (G) type hybrid breeding system of rye (*Secale cereale* L.)
17:40-18:00	Viktor Korzun Germany	Rye genomics and breeding: bridging the gap

June 22, 2021

	Session III	**Disease Resistance and Stress Tolerance**
	Chair: Andres Gordillo	
09:00-09:30	**Plenary Talk**	
	Thomas Miedaner Germany	Breeding for fungal disease resistances in small-grain cereals
09:30-09:50	Nikolaj Vendelbo Denmark	Discovery of a novel powdery mildew resistance gene on rye (*Secale cereale* L.) 7RL in a germplasm for hybrid breeding
09:50-10:10	Marvin Rose Germany	Correlation between inbred lines and testcrosses for resistance to *Fusarium* head blight in hybrid rye

10:10-10:30	Paul Gruner Germany	Mapping stem rust resistances in self-fertile and self-incompatible winter rye populations
10:30-10:50	Pawel Dopierala Poland	Artificial inoculation of *Claviceps purpurea* as an important tool for testing rye varieties for ergot resistance
10:50-11:10		**Coffee Break**
11:10-11:30	Anna Kodisch Germany	Reducing ergot susceptibility in rye for minimizing alkaloid contamination
11:30-11:50	Lev Tyryshkin Russia	Rye inbred lines susceptible to wheat leaf rust and genetic control of the trait
11:50-12:10	Vladimir Gorshkov Russia	Risk hidden under the snow: Psychrotolerant phytopathogenic fungi in Volga region of Russia
12:10-13:00		**Lunch Break**
13:00-14:30	**Chair: Andreas Börner**	**Meet the Sponsors/Elevator Pitch II**
	Session IV	**Rye for Food, Feed & Fuel**
	Chair: Thomas Miedaner	
14:30-15:00	**Plenary Talk**	
	Josef Kamphues Germany	Rye – great perspectives by its prudent use as feed and food
15:00-15:20	Tatiana Semilet Russia	Prospects for the creation of rye cultivars of universal usage
15:20-15:40	Ashifur Rahman Shawon Germany	Developing a crop model for simulating rye growth, development, and yield
15:40-16:00	Ludwig Riedesel Germany	Rye breeding promotes climate change mitigation
16:00-16:20	Rodrigo José Galán Germany	Integration of genotypic, hyperspectral, and phenotypic data to improve biomass yield prediction in hybrid rye
16:20-17:15	Andreas Börner/ Viktor Korzun	Discussion Round/Competion on location of next EUCARPIA RYE Symposium
17:15-17:30	Andreas Börner/ Viktor Korzun	Closing Remarks

Program Meet the Sponsors / Elevator Pitch I (June 21)

14:45-14:55	KWS	
14:55-15:05	Hybro	
15:05-15:15	Gergana Desheva Bulgaria	Morpho-agronomic assessment of genetic diversity among rye accessions using multivariate analysis
15:15-15:25	Vladimir Vasilievich Chaikin Russia	Change in winter rye yield and production process features during breeding
15:25-15:45	Elena Andreeva Russia	Transcriptomic analysis of violet colored and uncolored rye seeds
15:45-15:55	Maria Chiara Piro Belgium	Dissecting the genetic basis of arabinoxylan content in rye to provide new genetic resources for wheat breeding
15:55-16:05	Sabina Mehdiyeva Azerbaijan	Spike enlargement in *Triticale* through hybridization of branched and monstrosum ear donors

Program Meet the Sponsors / Elevator Pitch II (June 22)

13:00-13:10	KWS	
13:10-13:20	Hybro	
13:20-13:30	Hanna Bolibok-Brągoszewska Poland	Structural characterization and expression profiling of rye phosphate transporters
13:30-13:40	Elena Evtushenko Russia	Expression of *CENH3* genes in rye and *Secalotriticum* hybrid genomes
13:40-13:50	Liliya Gilmullina Russia	Genetic and selection approaches to assessing the content and structure of winter rye arabinoxylans
13:50-14:00	Tomasz Krepski Poland	Identification and characterization of putative brown rust resistance genes in rye (*Secale cereale* L.)
14:00-14:10	Mateusz Olechowski Poland	Does the gene *ScLr1* confer the resistance to brown rust?
14:10-14:20	Laura Valli USA	Ways of using rye to increase resilience and bring diversity to the field and the table

Content

Session I

Rye Genetic Resources and Diversity

Genebank2.0: Activation of the IPK winter wheat collection for breeding purposes - lessons learned and significance for pre-breeding in rye

J C Reif

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Plant genetic resources are the key to adapting crops to a changing climate, but their actual use to improve crops has remained limited. The general aim of the GeneBank2.0 project is to leverage the wheat ex-situ collection maintained at the IPK to an actively deployed one in breeding and research by applying an integrated concept encompassing cutting-edge genomics, phenomics, biodiversity informatics, and informed pre-breeding. The targets of our combined strategies for diversity exploitation range from single sequence polymorphisms to gametes with high value for wheat breeding. In the first phase of the project, the winter wheat collection was fingerprinted using genotyping-by-sequencing, and a trait-customized core collection was analyzed using resistance gene enrichment sequencing and whole-genome sequencing.

Intensive data mining based on the developed population and quantitative genetics pipeline resulted in donor accessions in the second phase of the project that have genomic regions promoting resistance to leaf rust, stripe rust and powdery mildew. Genome-wide prediction of breeding values for grain yield was performed within the IPK collection, but also across gene banks using the collection maintained at the Institut National de la Recherche Agronomique (INRA) as an example, and the most promising wheat accessions were selected. These donor accessions are forwarded to the breeding partners and integrated in their prebreeding programs. In addition, a select-and-backcross strategy was developed and implemented to unlock new diversity for flowering characteristics relevant for hybrid breeding. Initial phenotypic data are promising and highlight the potential to unlock useful variation for open pollination for elite wheat breeding from plant genetic resources. Promising donors are currently being used to develop doubled haploid lines that allow fine mapping of genomic regions that promote open pollination in wheat. The data generated within the GeneBank2.0 project is made available to all participating partners through a wheat information system in order to further stimulate the use the wheat ex-situ collection maintained at the IPK in breeding and research.

Not all bread is baked in one oven & not all rye is of simple descent

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As a minor crop with only local importance our understanding of the evolutionary history of domesticated rye and the relationship to its wild relatives is still limited. The history of cultivated rye (*Secale cereale* subsp. *cereale*) has long been mysterious, and many theories have been put forward as to its origin. The putative wild progenitor *Secale cereale* subsp. *vavilovii* still occurs in south-west Asia, as do those of its close relatives, wheat and barley. But in contrast to the latter two, rye was not used as a cereal crop until the European Bronze Age, several millennia after start of the Neolithic revolution. Hence, it is not among the founder crops of agriculture.

Archaeobotanical studies, genetic kinship analyses as well as the cultural history and etymology support the hypothesis of a secondary domestication origin from a weed in wheat and barley fields. In northern and central Europe, its exceptional winter hardiness and its tolerance to grow on poor soils, may have enabled the completion of the domestication process. Yet still, in some regions, e.g. Southwest Asia, rye grows as a weed among other cereals. Its ability to thrive on poor soils and the high frost tolerance enabled rye to become a suitable crop especially in the northern areas of Europe.

Combining the results from newly available genomic resources with archaeological, historic and linguistic evidence enables us to explore the differences in use and perception of rye in different geographic areas as a result of the underlying past processes.

Application of genetic resources for rye breeding in Volga Region

M Ponomareva[1], S Ponomarev[1], G Mannapova[2], L Gilmullina[2], L Ilalova[2], S Fomin[2],
N Garaeva[2], D Sayfutdinova[2]

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The main applications of grain are the bread baking, feed and alcohol industries. At the same time, different cultivars of rye are characterized by different suitability for the needs of a particular industry. The State Register of breeding achievements in the Russian Federation for 2019 includes 74 varieties and 10 hybrids of winter rye.

The aim of our work is breeding of high-yield, environmentally adapted and market-oriented winter rye varieties. Depending on the end uses there should be applied the contrasting selection criteria and variably resources in rye breeding program.

Decisive factors causing a decrease in rye yields in the Volga region are extreme winter conditions leading to crop damage. The reason for crop failure varies depending on the winter weather conditions, as a rule, the development of mushroom diseases under snow cover. The appearance of snow mold (*Microdochium nivale*) often leads to the most serious damage in susceptible varieties. Cases of appearance and damage of snow mold in winter are observed at intervals of 8 times in 10 years. The genetic resources of winter rye have been analyzed for resistance to snow mold. Screening of 90 samples was carried out from genetic resources of the collection of the All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov and own selection material of winter rye of Kazan Scientific Center both in natural conditions of defeat and at artificial introduction of pathogens into soil. For this purpose a unique experimental site was created - an infectious nursery, this for many years is exogenously enriched by pathogenic complexes of snow mold.

The applicability of grain for each purpose is largely determined by the content and structure of arabinoxylans. Water-soluble arabinoxylans from rye flour improve bread quality and have a beneficial effect on human health. At the same time, the high water holding capacity of these polysaccharides makes them an undesirable component of animal feed. Viscous solutions produced by arabinoxylans prevent digestion of feed. In brewing and alcohol production, gel precipitation of soluble and insoluble arabinoxylans causes a filtration problem, which increases time and financial costs. Thus, rye varieties differing in arabinoxylan content and solubility are necessary for various applications. We have examined more than 2500 samples and selected samples and lines for different directions.

Using a 140-year-old rye monoculture field trial to investigate how genetic and environmental factors affect genetic recombination

S Dreissig

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During sexual reproduction, novel allelic combinations arise via meiotic recombination, random assortment of chromosomes into gametes, and genetic exchange through cross-pollination. Allelic variants are in turn the targets of natural selection in changing environments, as well as selection by plant breeders for crop improvement. Selection in plant breeding is often hindered by low recombination rates (i.e. linkage drag). In the Triticeae, low-recombining pericentromeric regions comprise around 30% of all genes, rendering a significant part of the genome inaccessible to plant breeders. Therefore, increasing meiotic recombination rates holds promise to increase genetic gain in breeding programmes, and several key advances towards this aim were recently achieved in model species (*Arabidopsis thaliana*) and crops (*Brassica rapa* x *Brassica napus* hybrids, *Oryza sativa*, *Pisum sativum*, *Solanum lycopersicum*). Genetic recombination is also enabled by cross-pollination, which is important in hybrid breeding, and pollen size is critical for wind-pollination and seed setting in hybrids. Recombination is controlled at the genetic and epigenetic level, and shows plasticity towards environmental factors and availability of plant nutrients. A 140-year-old rye monoculture field trial conducted in Halle (Germany) provides a unique opportunity to investigate how genetic and environmental factors affect genetic recombination. By measuring differences in meiotic recombination rates and pollen size based on genetic divergence and under nutrient deficiency, we aim to understand the genetic achitecture mediating stress responses and interactions between genotype and environment. This will further our understanding of the processes involved in generating allelic diversity through meiotic recombination and cross-pollination.

Identification of factors that make the B chromosome of rye deviate from the Mendelian law of equal segregation

J Chen[1], A Boudichevskaia[1,2], M T Rabanus-Wallace[1], S T Nattar Amutha[1], A Houben[1]

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When transmission rates of chromosomes are higher than 0.5, not obeying the Mendelian law of equal segregation, the resulting transmission advantage is collectively referred to as 'drive.' Although supernumerary B chromosomes (Bs) possibly show the most common form of drive known for genetic elements, little knowledge exists about the molecular mechanism behind.

To decipher the process of drive we have selected the Bs of rye (*Secale cereale*) as a model. During the first pollen grain mitosis, B sister chromatids continue to stay cohesive rather than separate, and then both chromatids preferentially enter the generative nucleus. The drive process of rye B is (are) controlled by *trans-acting* factor(s) located at the end of the long chromosome arm of Bs, called non-disjunction control region (NCR). The deficient B (defB) chromosome lacks NCR and it undergoes normal disjunction at first pollen anaphase.

To decipher the *trans-acting* factor(s) responsible for the drive of Bs, we sequenced the mRNAs from the anthers of rye and wheat with standard Bs, defBs, or without Bs undergoing the first pollen mitosis. Combined with genomic PacBio CCS sequences of wheat carrying Bs, we assembled a reference transcriptome and performed a differential expression analysis by HISAT2-Stringtie-Salmon-DEseq2 pipeline. PCR-based mapping confirmed the physical position of seven candidates in the NCR of the rye B, the region encoding the *trans-acting* factor(s) of drive. Rye Bs of different geographical origins are able to drive. To test whether the same drive candidates exist irrespectively of their genotype, 0B/+B rye accessions from Japan, China, Korea, Turkey, Pakistan, and Afghanistan were tested. Regardless of their geographical origins, PCR-based mapping demonstrated that all candidates were B-specific. Thus, the identified candidates are likely essential for the function of the B. Next, the functional characterization of the candidate genes will be performed using the Virus-Induced Gene Silencing (VIGS) approach.

Session II
Breeding Methods and Molecular Tools

Rye genome sequenced - what`s next?

N Stein

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Two high-quality genome assemblies of rye were published recently. Both genotypes were selected for sequencing because of their importance for breeding programs in Germany (Europe) and China (East-Asia). Compared to previously reported genomic resources in rye the two recent assemblies provide, for the first time, straight access to all protein coding genes and, importantly, to their surrounding context of non-coding genome sequence, which is carrying the information for gene regulation. Together with the genome assemblies a few examples were provided to demonstrate the impact for traits analysis in rye. Hence, major limitations towards the full implementation of genome-informed breeding and research strategies could be removed. Given the high genome diversity that is characteristic for rye, however, there is a need to further improve rye genomic resources to facilitate the elucidation of important aspects of rye biology as well as for comprehensive access to genome-diversity information. The seminar will give an outlook into future rye genomics, providing also contigs of ongoing developments in the related cereal species barley and wheat.

Bridging the genotype-phenotype gap for SMART breeding in rye

B Hackauf

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With release of two high-quality genome assemblies, rye has finally reached the genome era, enabling the integration and advancement of fundamental and applied breeding and research to understand how the genome builds, maintains, and operates rye. Rye genome assemblies now enable association of the digital sequences of rye markers with locations in physical space as an essential standard to conduct and communicate genome-based breeding and research in rye. Despite of formidable achievements, major challenges in rye production remain, in particular concerning lodging-resistance and tailor-made grain qualities, to further advance rye from an all-rounder to an authentic high-performance crop with different and certified types of end-use. Physical mapping of genes and QTL controlling the unique reproduction biology of rye, agronomic traits, biotic and abiotic stress tolerance as well as grain quality in the 'Lo7' genome assembly will be presented. Using the male sterility inducing Pampa cytoplasm and the dwarfing gene *Ddw1* as an example, the options in rye for SMART (Selection with Markers and Advanced Reproductive Technology) breeding are illustrated. Semi-dwarf experimental hybrids are intended to advance the Green Revolution mainly by exploiting heterosis as well as by mobilizing a larger part of the total plant assimilates to the grain, which further improves the resource-use efficiency of rye. A highly diagnostic field phenotyping platform representing target environments for rye production in Europe and Canada will be presented to proof the practical potential of semi-dwarf as well as near-isogenic tall hybrids under potentially growth-limiting factors such as drought, frost, or nutrient deficiencies.

Genomic selection in rye hybrid breeding: from theory to practice

A Gordillo

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Before starting a genomic selection (GS) program, it is essential to characterize the genetic structure of the underlying prediction population to design the estimation population (EP) accordingly. The prediction population (PredP) may correspond to single crosses (families) comprising many lines or line panels derived from multiple families of small size. Prediction within single crosses allow for higher selection accuracies thanks to genetic relationship between EP and PredP.

In the KWS LOCHOW rye program we apply GS across line panels from multiple families. We classify our rye GS schemes according to the relationship between the EP and PredP. In specific, we differentiate whether the PredP is included in the EP (e.g. GS in parallel to combining ability tests) or not (e.g. GS at the line *per-se* selection stage prior to combining ability tests). As expected, our validation experiments show that the prediction ability for grain yield is considerably higher when predicted lines are included in the EP compared to independent EP and PredP. This proves a strong influence of the genetic background on marker-allele effect estimates.

When predicted lines are included in the EP, index selection combining both phenotypic and genomic estimated breeding values consistently delivers higher and more stable prediction abilities in validation experiments across years compared to phenotypic or genomic estimated values alone.

Although predictions based on independent EP offer lower prediction abilities, they still allow to considerably increase the probability of selection of lines with superior combining ability at the line *per-se* selection stage before combining ability testing.

Genomic prediction of yield in hybrid rye

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The main objective of this study was to investigate the potential for genomic prediction of yield in hybrid rye, using the genomic information from their parents/grandparents.

A total of 7,550 plots for 339 three-way hybrid rye were tested in the period 2016 to 2020 over 15 locations. There were 24 grandparent lines including 13 male sterile and 11 non-restorer, and 133 restorer lines crossed with the grandparent offspring lines. All the parents/grandparents were genotyped using 5K rye SNP array offered by TraitGenetics (Gatersleben, Germany). The genotypes of double mother offspring of grandparents' genotypes of three-way hybrid were imputed from the parental genotypes.

Estimation of variance components and model validation using leave-one-line-out cross-validation were carried out by using DMU software. The model included additive and dominance genomic effects and genotype by environment interactions with environments defined as location by year subgroups. The results showed that the additive genomic effects of restorer lines accounted for around 0.663 of the total phenotypic variances of three-way hybrid yield performance. The cross-validation was carried out for the restorer lines. The predictive ability, which is the correlation between three-way hybrid phenotype corrected for fixed effects and averaged over replicates and the genomic estimated breeding values of restorers, was 0.676.

In conclusion, it is possible to carry out genomic prediction of hybrid rye yield using genomic information from parents and the prediction accuracy is high. The results from the current study have significant implications for rye breeding. It is worthwhile to investigate other important traits in rye.

Breeding of winter rye hybrids at Federal Research Center "Nemchinovka"

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Winter rye as cross pollinated culture is very perspective for heterosis selection. However creation of heterosis hybrids of F_1 instead of varieties populations for the present did not become the dominating direction in the Russian breeding. In the Federal research center "Nemchinovka" of a works on creation homozygous of lines of rye are conducted on the basis of materials of from three genopul: Nemchinovka, Saratov and Vyatka. It is for this purpose applied repeated inbreeding the plants taken from the self-fertile populations received on the basis of these genopul. The backrossings for receiving sterile analogs and restorers of fertility on the basis of Pampas CMS are carried out. The assessment of restorer ability of these lines was carried out by visual survey of boots at hybrids from pair crossings. That genotype which at test crossings did not contain sterile and semi-sterile plants was considered as the best restorers of fertility (RI > 70%). In total more than 2000 homozygous lines of rye are studied. Many lines are rejected because of a weak rewintering, strong lodging and low productivity. On the basis of long-term data it is allocated about 350 rather highly productive, short-stem and winter-hardy lines. The general and specific combinational ability of lines was estimated in system diallel and the topcross of crossings. On the basis of these lines works on synthesis of simple and three-linear hybrids of F_1 with use of Pampas CMS are conducted.

Results of selection of the inbred lines necessary for synthesis of heterosis hybrids of winter rye are presented. Among examinees of lines the mfH-1432Rf and mfH-1423Rf lines were almost full restorers of fertility. On the basis of the inbred lines coming from these three genopul the perspective hybrid Nemchinovsky 1 is created. For years of competitive test (2017-2020) average productivity made it 6,4 t/hectare, having exceeded the Valdai standard on 0,63 t/hectare or of 10,9%. Advantage of a hybrid on productivity is reached due to bigger quantity of stalks (on 98 by 1 m). Average height of plants makes 136 cm that up to the standard. Thanks to stronger stalk the hybrid has the best resistance to lodging and on winter hardiness and mass of 1000 grains is up to the standard. On traits of quality of grain (amilogramma height, falling number, starch pasting temperature, viscosity of water extract of grain meal, fluidity of toppling bread) hybrid has the best indicators than the standard. The new hybrid is transferred to the state test. Problematic questions of profitability and the organization of seed farming of hybrid varieties of rye are discussed.

Hybrid rye in Belarus: breeding results, cultivation

E Urban, S Hardzei

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Thirty-two winter rye cultivars are included in the State Register of Varieties of the Republic of Belarus for 2020, of which 16 cultivars are of Belorussian breeding: 15 population cultivars and one F_1 hybrid Plisa. Also 14 F_1 hybrids of German breeding were registered: Picasso, KWS Bono, KWS Ravo, KWS Livado, KWS Binto, KWS Vinetto, KWS Dolaro, KWS Serafino, SU Drive, SU Mefisto, SU Cossani, SU Performer, SU Bendix, SU Forsetti, and two joint F_1 hybrids (KWS Lochow GmbH & Scientific and Practical Centre of Belorussian NAS for Arable Farming) LoBel 103 and Galinka.

The new our F_1 hybrid Belgi has been studying in State Variety Testing.

Cultivars of Belorussian breeding in 2020 occupy 92% (338 382 ha) of winter rye sown areas in the Republic, 8% (29 053 ha) are occupied by F_1 hybrids of German breeding and 0,15% (557 ha) – by Belorussian F_1 hybrid Plisa.

Belorussian F_1 hybrids Plisa and Belgi were developed on the basis of the CMS "Pampa". Plisa is a linear-population hybrid; Belgi is a simple interline hybrid. CMS "Gülzower" is also used in the breeding program, however, till today it has not been possible to develop a hybrid cultivar based on this type of CMS because of the extremely low frequency of non-restorer genes in rye populations.

It is known that for the fullest realization of the genetically determined productivity potential of F_1 hybrids, richer soils and a high level of cultivation technology are required in comparison with the requirements for population cultivars. On poor sandy soils, F_1 hybrids do not increase productivity over population cultivars. In Belarus about 44% (2 million ha) of arable land are poor sandy soils spread by sand with low level of natural fertility, unstable water mode and high level of acidity. This is the main reason hampering the cultivation of hybrid rye in Belarus.

Today only economically strong farms, that have reached yields of 45 dt/ha or more, can have high profitability for cultivating rye F_1 hybrids. However, in the nearest future, the share of such farms will increase and the area under rye hybrids, according to our estimates, may amount to about 45-55 thousand ha, or about 12-17% of all rye area in Belarus.

Site-directed genetic engineering in cereals - principles and applications

J Kumlehn

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Methods of site-directed genome modification offer great opportunities for fundamental plant research and the improvement of crop performance. As a precondition for keeping pace with the rapid development of CRISPR-associated (Cas) endonuclease technology, a modular and versatile vector system has been developed. Being based upon the use of type IIS restriction enzymes, it allows for complex molecular cloning procedures comprising just a few comparatively simple steps. Not only can multiple guide RNAs be expressed simultaneously, also newly emerging system components such as Cas derivatives with improved or novel functionality can be readily tested and utilized. The increasing interest in customizable endonuclease technology is also associated with a renaissance of protoplast culture and transfection, which is of great utility for the development of novel methodical approaches as well as for the functional validation of system components before it comes to the exceedingly laborious targeted genetic modification at the whole-plant level. In addition, options to couple site-directed genetic engineering with haploid technology have been implemented, be it for an efficient genetic fixation of modified alleles or for the use of *cas9*/gRNA-transgenic haploidy inducer lines for cross-pollination, which has the capability of triggering target site-specific modifications with much reduced genotype dependence. Recent results of translational research in barley and wheat include the establishment of plant resistance to pathogens and the modification of further yield- and quality-determining plant features. Since it has not yet been possible to establish implementable haploid and transformation technologies for rye, there is unfortunately not yet a single example of the application of customizable endonucleases for this agronomically and nutritionally important cereal. However, the progress achieved with other Triticeae crops and the interim results of ongoing work give good reason to hope that site-directed genetic engineering will also be possible for rye in the near future.

Establishment of adventitious shoot formation *in vitro* and a genetic engineering platform for rye

D E S Daghma, L Lorenz, C Marthe, S Knuepffer, J Kumlehn

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Rye (*Secale cereale* L.) is a diploid winter or spring cereal plant which is highly recalcitrant to plant regeneration *in vitro* and genetic transformation. It is genetically and phenotypically highly versatile and the second most used crop to produce bread worldwide, while green plants are used for livestock pasture. Rye is widely used as a gene pool for improving wheat because of its high tolerance to harsh conditions such as drought, low nutrient availability, and cold temperatures, which makes it a particularly useful crop where other species fail to be productive. In addition, rye can be grown as a cover plant to prevent soil erosion. In contrast to its high value, rye plants suffer considerable damage by several diseases such as ergot, while biotechnologies such as haploid technology and genetic engineering do not yet play a role in current breeding programs. To provide a technological bases for genetic engineering approaches by establishing the principle of adventitious shoot formation from immature embryo explants, multi-factorial experiments were conducted including 12 rye genotypes, five callus induction media and 3 regeneration media.

Callus induction, callus type, and regeneration ratio were highly affected by genotype and culture conditions. Inbred line Lo7, which represents the sequenced reference genome, showed the highest percentage of callus induction and plant regeneration. Based upon these results, genetic engineering using Agrobacterium-mediated or biolistic DNA delivery is in progress to produce stable transgenic and genome-edited rye plants.

Genomic scan of male fertility restoration in Gülzow' (G) type hybrid breeding system of rye (*Secale cereale* L.)

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The key to harnessing hybrid vigor or heterosis in rye (*Secale cereale* L.), is efficient restoration of male fertility (*Rf*). In hybrid rye breeding, the Gülzow (G) type cytoplasmic male sterility (CMS) mechanism exhibits a powerful and environmentally stable restoration of male fertility. Using a comprehensive set of genomic methods and taking advantage of the recently released reference genome of rye, we report a thorough investigation of genetic mapping and scanning for restoration of male fertility in a broad G-type CMS breeding system. The genomic tools includes (i) genome wide association studies (GWAS) on a G-type germplasm, (ii) GWAS on a biparental mapping population, (iii) scanning of rye genome for the identification of probable candidate genes and their expression pattern in G-type rye hybrids, and (iv) mining patterns in linkage disequilibrium. Our findings provide compelling evidence of a novel major G-type *Rf* gene on the 3RL chromosome and shed light on the genetics of male fertility restoration in a G-type CMS system in rye.

Rye genomics and breeding: bridging the gap

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Great advances have been made in recent years in systems to detect DNA variation and in the technologies used to identify DNA markers linked to useful traits. More recently, single nucleotide polymorphism (SNP) markers have been developed for rye and this marker system is predicted to accelerate advances in both marker development and implementation in breeding programs. Identification of markers linked to useful traits has been based on complete linkage maps, genetic mapping or/and on whole genome wide association studies (GWAS). Use of genomic selection (GS) can greatly speed up the selection process and increase precision. In the presented work, the development of molecular genetic maps, application of molecular markers for characterisation of genetic resources, identification of abiotic and biotic stress tolerance loci in rye and selected applications in breeding are presented and discussed. Future perspectives for public-private collaborations in rye research and breeding will be highlighted.

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Session III

Disease Resistance and Stress Tolerance

Breeding for fungal disease resistances in small-grain cereals

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Small-grain cereals are attacked by a plethora of fungal diseases. Powdery mildew, diverse rusts and Fusarium head blight (FHB) are attacking all of them, Septoria tritici blotch is specially adapted to wheat, net blotch and Ramularia leaf spot to barley and ergot is mainly a disease of rye. Triticale is nowadays one of the most susceptible cereals attacked by both, wheat and rye pathogens. In view of fungicide resistances, restrictions in application and registration of new fungicides in the European Union and rising costs for the farmers, resistance breeding is more important than ever. A study on the breeding progress in the last 33 years in German winter wheat shows that selection gain was largest for resistances to Septoria tritici blotch, powdery mildew, leaf rust, and lowest for yellow rust (Laidig et al. 2021). However, leaf rust and powdery mildew have by far the largest age trends making the resistances less effective during the lifetime of a cultivar, while FHB resistances remain stable across >20 years. Comparing large assortments of old and new European wheat and triticale cultivars clearly showed a qualitative inheritance for yellow rust and stem rust resistances and a quantitative inheritance for FHB resistance with about 10% of the cultivars displaying a multi-disease resistance (MDR). Typically, all quantitative disease resistances are caused by hundreds of QTLs scattered across the whole genome, but often available in hotspots. A genome-wide association mapping study revealed that MDR might be governed by additional loci that are not detected when analyzing one of the disease resistances only (Miedaner et al. 2020a). Finally, the integration of genomic data into the breeding process for introgression of genetic resources and for the improvement within elite materials is discussed (Miedaner et al. 2020b). Genomic selection procedures should be successful for improving cultivars for MDR in wheat and should be applied also to other cereals.

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Discovery of a novel powdery mildew resistance gene on rye (*Secale cereale* L.) 7RL in a germplasm for hybrid breeding

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Ranking amongst the top 10 most damaging crop diseases globally, powdery mildew (PM) is capable of inflicting considerable grain yield and quality losses in cereals. In rye (*Secale cereale* L.), PM is caused by the ascomycete fungus *Blumeria graminis* f.sp. *secalis* (*Bgs*), which is prevalent in rye growing regions. Recognized a generally high level of resistance to biotic stresses, rye has historically served as a significant source for wheat improvement for disease resistance by chromosomal translocation of segments harboring PM resistance genes. At present seven major PM resistance genes have been identified in rye, *i.e. Pm8*, *Pm17*, *PmCn17* (1RS), *Pm7* (2R), *Pm?* (4R), *Pm56* (6RS) and *Pm20* (6RL). Here, we report a PM resistance gene in a hybrid breeding germplasm. In total, 180 inbred rye lines from Nordic Seed Germany GmbH were phenotyped in greenhouse using three distinct *Bgs* populations originating from Denmark and Germany. Lines were genotyped using the high-density 600K SNP array, producing 261406 polymorphic SNPs. Genome-wide association nalysis revealed a singular peak on the distal region of the 7RL chromosome. In close proximity to the PM-resistance associated locus, a cluster of three full-length nucleotide-binding leucine-rich repeat (NB-LRR) genes have been annotated in the 'Lo7' rye reference genome. Our finding constitute a novel discovery in the field of rye PM resistance with little known on orthologous genes in syntenic regions on barley 2HS and wheat 2AS-BS-DS.

Correlation between inbred lines and testcrosses for resistance to *Fusarium* head blight in hybrid rye

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Hybrid breeding is based on selection of inbred lines in early generations. A sufficient genetic correlation between lines and testcrosses is, however, the prerequisite for a higher selection gain in the hybrids. Therefore, we investigated this crucial parameter for FHB resistance on 60 lines each of the heterotic groups Petkus and Carsten and their corresponding crosses with two testers each at four (lines) or six (testcrosses) environments (location $\times$ year combinations). FHB severity measured as percentage of infected spikelets per plot was considerably higher for the lines than for the testcrosses. A correction was made for a correlation between FHB resistance and flowering time by using the latter as covariate. As expected, the lines had twice as much genetic variance as the testcrosses, the genotype $\times$ environment interaction was significant in both materials ($p \leq 0.001$), and the entry-mean heritabilities ranged from 0.78 to 0.88. For both lines and testcrosses, moderate negative correlations with plant height (r=-0.21 to -0.55) were found. The genetic correlation between line and testcross performance was r=0.76 for the Petkus and r=0.69 for the Carsten heterotic group. In conclusion, this correlation allows a selection on line basis; in the last generation before the Official Trials, testcrosses should also be tested for FHB resistance and mycotoxin content to reliably improve food and feed quality in rye.

Mapping stem rust resistances in self-fertile and self-incompatible winter rye populations

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Stem rust (*Puccinia graminis* f. sp. *secalis*) is an important disease in winter rye (*Secale cereale* L.). To trigger the start of resistance breeding we analyzed (A) populations composed of recombinant inbred lines developed by crossing a resistant single plant with a susceptible elite breeding line and (B) pre-selected genetic resources comprising a random sample of self-incompatible plants. Both were genotyped by a 10k SNP chip. For (A) phenotypic data was collected from multi-environment field testing in Germany and Poland accompanied by leaf-segment tests. QTL mapping revealed a single *R* gene for all-stage resistance in two populations and several QTL for adult-plant resistance in the third population. Five genetic resources with about 70 single plants each of (B) were analyzed by a leaf-segment test that allow testing several rust isolates for the same plant simultaneously, thus detecting, however, only all-stage resistances. Statistical methodology for categorical data was applied and could reveal new loci for *R*-gene candidates. However, unbalanced ratios of resistant and susceptible plants in the genetic resources reduced the power of significance tests. Promising SNP marker candidates were transferred into single KASP assays to be further used in practical breeding. From the view of durability, the new *R* genes should only be used in a pyramided manner or complemented by the detected QTL.

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Artificial inoculation of *Claviceps purpurea* as an important tool for testing rye varieties for ergot resistance

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Ergot, caused by the fungus *Claviceps purpurea*, is a severe disease of rye. The fungus replaces the grains with a dark, purplish mass of mycelium called sclerotia. The disease can lead to considerable economic losses due to the contamination of the grain with ergot alkaloids that are toxic for humans and animals. At present, the E.U. thresholds for ergot are 0.1% by weight of sclerotia in the harvest ware intended for animal feed and 0.05% for human consumption. From the year 2021, ergot alkaloids threshold of 500 µg/kg for ergot for human consumption will be introduced for the first time. Moreover, the E.U. Commission is discussing the introduction of more stringent thresholds for ergot sclerotia from the year.

Since rye is a cross-fertilizing crop, it is particularly susceptible to ergot infection. Usually, population rye varieties show a higher tolerance to ergot than hybrid varieties because of the hybrid system based on cytoplasmic male sterility (CMS). Major genes for the restoration of pollen shedding can be introgressed into rye hybrids, thus leading to a substantial reduction of ergot infection. Hybrids developed with this new technology are marketed by KWS under the brand PollenPlus®

During the registration process of the Research Center for Variety Testing (COBORU), resistance to diseases is normally assessed. In the past, COBORU assessed the amount of ergot sclerotia in the harvest ware of individual candidate varieties under natural infection conditions. Unfortunately, often the level of natural infection is very low and, therefore, no differentiation for ergot susceptibility is realized. In the harvest season 2020, special ergot trials were conducted for the first time at two COBORU locations using an artificial infection methodology in agreement with that of the German variety office (Bundessortenamt). Rye candidates in the second and third year of official trials were tested together with check varieties. In specific, ergot spores were sprayed shortly before, during, and after flowering time in two-day intervals. This way, the level of ergot infection and repeatability of ergot trials could be improved considerably compared to natural infection conditions. Significant differences were found in 2020 between the tested varieties. These results were well correlated with those of KWS ergot trials with artificial infection

On this basis, we conclude that ergot resistance trials with artificial infection provide more reliable information to assess the ergot susceptibility of rye varieties. This is an important tool to characterize rye varieties regarding their ergot resistance, which is becoming increasingly important in the frame of new, more stringent, ergot thresholds by the E.U. We recommend continuing and extending the realization of artificial ergot trials by COBORU.

Reducing ergot susceptibility in rye for minimizing alkaloid contamination

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Ergot caused by *Claviceps purpurea* prefers cross-pollinating grasses such as rye (*Secale cereale* L.). After an infection, alkaloid containing black-purpled sclerotia are formed on the ear of the host plant. Four factors influence ergot reaction: pollen availability, restorability of the female, morphological characteristics that lead to disease escape, and possibly a physiological resistance mechanism. Strict limits are established within the European Union, and a further reduction in near future is already in discussion. For that reason, 16 winter rye cultivars, two factorial crossing designs with each of five female and five male lines differing in their restorer genes and 10 CMS-single crosses were evaluated by artificial inoculation in up to 20 environments in a tri-national CORNET project. We analyzed 12 ergot alkaloids (EA) of 372 diverse field samples by HPLC considering genotypes, isolates, locations and years. High and significant genotypic variation regarding ergot severity and pollen-fertility restoration were observed. The pollen fertility restoration of the male had the highest impact on ergot severity, but also the female component revealed a significant effect, even under pollen isolation. A huge influence of location and year was detected for EA content. A moderate, positive covariation between total alkaloid content and ergot severity was observed, but large deviation from the regression were detected. Therefore, a reliable prediction of the total alkaloid content based on ergot severity is at present not possible. In conclusion, further improvements of ergot resistance by selecting superior restoration ability is still the most promising way, but also females with lower ergot receptivity could be selected in future breeding programs.

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Rye inbred lines susceptible to wheat leaf rust and genetic control of the trait

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According to current scientific concepts, vast majority of rye genotypes are very resistant (immune) to the highly specialized pathogen of wheat leaf rust *Puccinia triticina*. In this study we describe the susceptibility of several rye inbred lines to natural *P. triticina* population at seedling stage. For line L393 the susceptibility was shown also at flag-leaf stage. To study genetic control of the trait this line was crossed with resistant L371. Segregation in F_2 and in progeny of F_1 crosses with wheat Chinese spring has been analyzed. Hybridological analysis revealed that 1. this unusual susceptibility at seedling stage is the recessive trait; 2. cytoplasm has no or minor influence on the phenotypic expression of the susceptibility; 3. the trait is controlled by 3 complementary genes (2 dominant and 1 recessive). This genetic control indicates that when the L393 line is involved in rye breeding, the probability of the appearance of plants susceptible to wheat leaf rust in the F_2 generation will be relatively high; wide use of L 393 in rye breeding could result in a serious threat to the crop by this pathogen. As expected, primary triticale developed on the bases of L393 was susceptible to *P. triticina* infection.

Risk hidden under the snow: psychrotolerant phytopathogenic fungi in Volga region of Russia

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Snow mold disease leads to high losses of winter cereals. This disease is usually caused by different psychrotolerant phytopathogenic fungi from ascomycetes (*Microdochium nivale* and *Sclerotinia borealis*) and basidiomycetes (*Typhula ishikariensis* and *T. incarnata*). Although different snow mold-causing fungi are from distant phylogenetic groups, their "resemblance" with each other is determined by the ability to cause disease under the snow cover or right after its melting.

In Tatarstan region (Russia) and some regions of the Volga Federal District, in 2019, there was an epiphytotia of snow mold that forced the initiation of a comprehensive multidisciplinary project directed towards the study and control of this disease. Snow mold-causing fungi are very poorly investigated. In particular, no virulence factors including extracellular enzymes were described for them and we know almost nothing about the pathogenesis caused by these fungi. All these form a significant obstacle for the design of different plant protection approaches.

We collected the isolates of snow mold-causing fungi (*Microdochium nivale*, *Typhula ishikariensis*) colonizing diseased winter rye plants in Tatarstan. The isolates were characterized in terms of their growth, morphology, extracellular enzymatic activities and their phylogenetic position was determined on the basis of ITS sequence. Our results show that snow mold-causing fungi parasitize as pathocomplex represented by different varieties of the same species characterized by different phenotypes and virulence properties.

The first results about pathogens and an integrated research and strategies against snow mold will be presented and discussed.

Session IV
Rye for Food, Feed & Fuel

Rye – great perspectives by its prudent use as feed and food

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Cultivation of cereals to produce feed and food is facing diverse challenges (climate change, impact on the environment, consumers` expectations, animal welfare). Prudent use of rye means that its typical properties are used specifically to maximize the benefits for the farmer and producers of feed and food as well as for consumers (humans / animals). Similar to other cereals, rye has a high starch content, whereas its protein content is slightly lower than in wheat or oats. However, rye has a unique composition regarding its non-starch polysaccharides: mainly arabinoxylans and fructans contribute to its high dietary fibre content. In monogastric species (including humans and animals), consumption of rye results in a unique effect, i.e. the favoured supply of the hind gut with highly fermentable substrates. The subsequently stimulated microbial formation of butyric acid (a volatile fatty acid) can explain diverse beneficial effects within and outside the alimentary tract. Rye is also characterised by the highest phytase activity among all cereals (phosphorus utilization in monogastric species). On the other hand, rye harvest ware may carry ergot sclerotia that contain toxic alkaloids. However, the risk of ergot contamination can be lowered to a minimum by appropriate agronomic management and rye varieties with superior pollen-shedding ability. On the positive side, the burden of rye by Fusarium toxins is markedly lower than in corn or wheat. From the view of digestive physiology, rye is not only an energy and starch source, it is rather suitable to favour gut health and wellbeing by its particularly positive effect in the gastrointestinal tract.

Prospects for the creation of rye cultivars of universal usage

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Rye is one of the few crops that has become widespread in Russia and abroad. Due to its highly adaptive, rye can be grown on sour and infertile soils as well as has high tolerance to drought and low temperatures. In addition, the rye contains a large amount of carbohydrates, proteins and vitamins related to A, B and PP groups. Nowadays rye cultivars content a large amount of essential amino acids, such as lysine and threonine. These ones are intended to increase the nutritional and feed value of modern rye cultivars.

However, to date the rye demand is not high in Russian and foreign countries. In Russia, it uses in baking, brewing and distillation industries. The usage of rye grain is limited in fodder production because of higher five-carbon sugars content such as water-soluble pentosans (arabinoxylans) in rye by comparison with wheat in 3-4 times.

Water-soluble arabinoxylans (WS-AX) are found in cell walls, intercellular spaces of seed coats and in the aleurone layer. Being a polymer with a high molecular weight, these carbohydrates display highly viscosity in solutions and limit the access of enzymes to the grain nutrients that leads to digestion disorders in animals. In addition, the animal's enzyme system does not contain the enzyme hydrolyzing pentosans. The problem is solved by adding the enzymes with pentosans hydrolyzing activity to main diet. However, this approach leads to insufficient hydrolysis, side effects and the price of feed increases, so that it solves the problem partially.

Therefore, the development of rye cultivars with a low content of WS-AX is an important issue. The method of low-pentosan forms isolation from the rye without a biochemical analysis has been developed in VIR. It is based on the anatomical and morphological traits of the grains which differ the content of water-soluble pentosans. The approach allowing select grain with an extremely low (0.5-0.9%) content of pentosans has been developed. Six low-pentosan rye cultivars were developed using this method. Grain of low-pentosan rye varieties surpass all grain crops due to special nutritional properties and can be effectively used in animal diets without enzyme additives.

Developing a crop model for simulating rye growth, development, and yield

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Rye (*Secale cereale*) has its wide acceptability due to a high resource use efficiency at low input levels, hardiness against frost and drought, and provision of healthy grain. Introgression of the gibberellin (GA)-sensitive dwarfing gene *Ddw1* in hybrid rye intends to improve lodging tolerance and harvest index as well as carbon footprint of rye production. Process-based dynamic crop models are cutting-edge tools in current agronomic research by simulating crop growth, development, and yield under diverse soil, climate, and management conditions. They enable to evaluate the impacts of climate variability and management practices on agricultural production beyond limited field experiments. Using available long-term multi-site experimental data of released population and hybrid rye varieties, an existing wheat model is currently adapted for rye. Morpho-, physio-, and phenological parameters characterizing tall and semi-dwarf rye genotypes will be adjusted based on multi-environmental trial data. The model will serve to simulate numerous "virtual experiments" testing the performance of tall and near-isogenic semi-dwarf genotypes in diverse environments under current (1991-2020) and future (2035-2065) climatic conditions. Predicting complex phenotypes with G×E as function of genotypic and environmental inputs will support breeding programs to further optimize the performance of rye in various target environments in a changing climate.

Rye breeding promotes climate change mitigation

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Agriculture contributes to about 25% of total anthropogenic greenhouse gas emissions (GHG), which are projected to further increase in the future. Little is known about the effect of modern crop varieties on climate change mitigation (CCM). In this study, we assessed greenhouse gas emissions per unit land (GHG) and carbon footprint per unit harvest product (CFP) of winter rye and winter wheat from official German variety trials during 1983 and 2019. The data set refers to released rye (n=94) and wheat (n=359) varieties, which were each tested for three years at 15-30 locations per year throughout Germany. All rye varieties were evaluated at two intensity levels, i.e., with vs. without fungicides, and breeding types, i.e., hybrid vs. population varieties. We conduct a partial life cycle assessment and analysed the data using mixed models. GHGs in rye were about 10% higher with fungicides than without. Moreover, hybrid varieties revealed 4% higher GHG emissions than population varieties. Furthermore, breeding progress let to slightly increasing GHG emissions of ~+4-10% since 1983. However, considering CFP we observe an opposite trend, where fungicide treated varieties reveal 8-10% lower CFPs than varieties without fungicide treatment and hybrid varieties reveal 9% lower CFPs than population varieties. Moreover, the breeding progress of rye leads to exponentially decreasing CFPs by ~–13-17% since 1983. Notably, rye revealed about 20% lower GHG emissions and 9% lower CFPs as compared to wheat. We consider the significant contribution of rye breeding progress to reducing CFPs as a valuable contribution to CCM.

Integration of genotypic, hyperspectral, and phenotypic data to improve biomass yield prediction in hybrid rye

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Integrating cutting-edge technologies are imperative to sustainably breed crops for a growing global population. To predict dry matter yield (DMY) in winter rye (*Secale cereale* L.), we tested single-kernel models based on genomic (GBLUP) and hyperspectral reflectance-derived (HBLUP) relationship matrices, a multi-kernel model combining both matrices and a bivariate model fitted with plant height as secondary trait. In total, 274 elite rye lines were genotyped by a 10k-SNP array and phenotyped as testcrosses for DMY and plant height at four locations in Germany in two years (= eight environments). Spectral data consisted of 400 discrete narrow bands ranging between 410 nm and 993 nm collected by an unmanned aerial vehicle (UAV) on two dates on each environment. To reduce data dimensionality, variable selection of bands was performed, resulting in Lasso as the best method in terms of predictive abilities. The mean heritability of reflectance data was moderate (h^2=0.72) and highly variable among the spectrum. Correlations between DMY and single bands were generally significant (p<0.05) but low (≤0.29). Across environments and training set sizes (TRN), the bivariate model showed the highest prediction abilities (0.56-0.75), followed by the multi-kernel (0.45-0.71) and single-kernel (0.33-0.61) models. With reduced TRN, HBLUP performed better than GBLUP. HBLUP fitted with a set of selected bands was preferred. Within and across environments, prediction abilities increased with larger TRN. Our results suggest that in the era of digital breeding, the integration of high-throughput phenotyping and genomic selection is a promising strategy to achieve superior selection gains in hybrid rye.

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POSTER

Morpho-agronomic assessment of genetic diversity among rye accessions using multivariate analyses

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The aim of the study was to assess the genetic diversity of 27 rye (*Secale cereale* L.) accessions by using multivariate statistical methods. The experiment was carried in the block method with with 3 replications on plots of 2.5 m during 2017/2018, 2018/2019 and 2019/2020 growing seasons. Nine qualitative and fifteen quantitative morpho-agronomical characters were evaluated. General Shannon-Weaver diversity index for all included in the study traits was moderate (H'=0.631). The analysis of variance exhibited the presence of significant differences among the tested genotypes for all characters indicating the existence of variability. The high heritability with high genetic advance was recorded for Leaf area of a flag leaf, leaf area of a sub-flag leaf, weight of the main spike, weight of the grain in the main spike, number of spikelets in the main spike and grain yield combined indicating that they are important characters to be considered for selection and improvement of Secale cereale L. Cluster analysis based on the Between groups linkage and Squared Euclidean Distance grouped the genotypes into four clusters. The highest distance values were measured for the following pair wise genotype combinations: B4000206-B7000123 and B4000205-B7000123. The first 8 principal components explained a very large proportion of the total variation (81.80% %). The results of the study will be useful for the breeding improvement programs of rye.

Change in winter rye yield and production process features during breeding

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In order to search for traits suitable for breeding of highly productive genotypes and developing a varietymodel, the yield and characteristics of the production process of the old and modern varieties of winter rye created at the institute with an interval of 80 years, grown under the same conditions were compared. It was found that the yield during this period of selection had increased by 1.55 times. Its growth was due to a sharp (by 60.6%) increase in the specific cenotic productivity, the number of grains over an area (by 20.2%) and the yield index (by 6.5%). The coenoses of the varieties compared differ in structure. The modern variety has more shoots per area unit and bigger shoot mass. Unlike the old variety, the cenosisof the modern variety is highly resistant to lodging. It has a 33.5% higher leaf area index and a 17.7% higher upper layer leaf mass. According to the content of total chlorophyll in dry matter of leaves and sheaths, the modern variety is inferior to the old one during earing by 30.2%, and during milky-wax ripeness – by 17.5%. The varietiescompared practically did not differ in the content of total chlorophyll in the sown area, but the ratio between chlorophyll *a* and *b* changed noticeably. Chlorophyll *a* predominated in the earing phase, and chlorophyll *b* predominated during the milky-wax stage. In terms of content of chlorophyll in the stems, both in dry matter and in the sown area, the modern variety was 1.5 and more times inferior to the old variety in both record periods. The varieties under study differed in the biomass accumulation and its distribution between the organs during the growing season. The old variety always used only the products of the current photosynthesis to form the productivity of an ear. The modern variety, in the conditions of an unfavorable year for growth, thanks to re-utilization, used for this the assimilates previously accumulated by the leaves. The real and potential productivity of the ear of the modern variety is higher, respectively, by 77.4 and 68.0%, but the degree of its provision with vegetative mass is by 48.6% less. Only due to the 77.4% higher shoot mass utilization coefficient, the modern variety sets a larger number of grains in the ear with a larger mass of each. Since a sharp increase in the productivity potential of the ear was not accompanied by the same growth in the shoot mass, the modern variety had a disturbed balance in the "vegetative shoot mass - filling grain" system. In unfavorable conditions for growth, it can be the reason for the instability of the yield level achieved as a result of breeding. It is necessary to search for a more acceptable morphotype of the rye plant.

Transcriptomic analysis of violet colored and uncolored rye seeds

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The Peterhof genetic collection of rye maintains lines with different types of grain color. The violet color of the pericarp is presumably controlled by the Vs gene (violet seed). Since there is a variability in seed color from an intense violet color ("strong" dominant allele) to a weak violet color - ("weak" allele) and lack of color (recessive allele vs), we assume that the Vs gene is a transcription factor that controls the work of structural genes for the synthesis of anthocyanins.

Transcriptome analysis of the pericarp and aleurone of caryopses of rye lines (12 days after pollination) with varied seed color revealed interline differences in the expression of genes involved in the synthesis and regulation of anthocyanin synthesis: the "late" genes for anthocyanin synthesis, genes of transcription factors that are homologues of maize genes Anthocyanin regulatory RS protein and Anthocyanin regulatory C1 protein. The number of transcripts between violet and uncolored lines significantly differ and is higher for stained lines, which allows us to consider these genes as candidate genes Vs.

Dissecting the genetic basis of arabinoxylan content in rye to provide new genetic resources for wheat breeding

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Genome wide association studies (GWAS) constitute a powerful approach for uncovering the genetic basis of quantitative traits. A quantitative trait for which there is growing interest is cereal dietary fibre (DF) content. A promising target to enhance the DF content in refined flours is arabinoxylan (AX) since it constitutes the major DF component in the endosperm of cereals such as rye (*Secale cereale*) and wheat (*Triticum aestivum*). A preliminary screening by the FIBRAXFUN consortium on rye and winter wheat cultivars highlighted that the AX content of rye wholemeal ranges from 5.9 to 8.6%, whereas in wheat it amounts to only 4.2 to 5.3%. To dissect the genetic basis of AX content in rye, we have assembled a diverse germplasm panel consisting of 312 accessions of winter (294) and perennial rye (18). The panel comprises 289 *S. cereale* accessions (subsp. *cereale*, subsp. *afghanicum.*, subsp. *ancestrale*, subsp. *dighoricum*, subsp. *segetale*), 18 *S. strictum* accessions (subsp. *scrictum*, subsp. *anatolicum*, subsp. *kuprijanovii*), two *S. sylvestre* accessions, one *S. vavilovii* accession, and two hybrid accessions (*S. cereale × S. vavilovii*, *S. vavilovii × S. cereale*). These accessions include landraces and cultivars of diverse geographical origin, with 300 of them originating from 37 Eurasian countries, 11 from five countries in the American continent and one from South Africa. The collection will be genotyped following a Genotyping-By-Sequencing approach and phenotyped with regards to AX content. These data will be combined in a GWAS to uncover the genetic determinants of AX content in rye kernels and establish the foundation for the introgression of AX-associated rye loci into wheat.

Spike enlargement in *Triticale* through hybridization of branched and monstrosum ear donors

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The formation of supernumerary spikelets in cereals is considered as one of the concepts for the affecting of grain yield. In our work two hexaploid triticale lines – ABR (genome AABBRR) and PRAG 206 (genome AABBRR with the 2R/2D chromosome substitution and 4B:6B translocation), carrying genes for the "branched spike" and "monstrosum ear", subsequently, were involved in hybridization. The goal of this project was the generation of recombined material from these two lines for selection of plants with enlarged spikes. The plants with the enlarged spikes, that resembling mainly the monstrosum ear, but with the more stronger branches in the lower or most part of spikes as in the case of turgidum type of branching, were obtained in F_3 generation. Enlargement of spikes is resulted by strong bending of peduncle in these plants, that requests the further crosses for its improvement. FISH analyses of plants from this cross having the small-sized monstrosum spikes and erect peduncle revealed chromosome translocation, presumably 2A:2R.

Structural characterization and expression profiling of rye phosphate transporters

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Phosphorus (P) deficiency is one of limiting factors for crop production, especially on acidic soils, which constitute ca. 50% of arable land on Earth. Rye (*Secale cereale* L.) is a cereal with an exceptionally good tolerance of biotic and abiotic stresses, including nutrient deficiency stress. Therefore, it is an excellent object for research on P deficiency tolerance related genes, which might deliver interesting candidates for crop improvement – development of more P-efficient plants. To date several classes of P uptake, allocation and deficiency response related genes and gene families were identified in several crop and model plants, however, there is no data on genetic control of P use efficiency in rye. We searched the two available rye genome reference sequences of Lo7 and Weining and a BAC library of rye inbred line L318 and identified multiple putative rye phosphate transporters. We performed a comparative analysis of their structural features and a phylogenetic analysis involving sequences phosphate transporters from other plants. The involvement of identified phosphate transporters in P deficiency response was verified using qRT-PCR and root and shoot tissues of rye inbred lines subjected to P-deficiency (0.01 mM KH_2PO_4, hydroponic conditions, early stages of vegetative growth). Obtained results provide foundation for further studies - functional characterization and sequence diversity screens in germplasm from different cultivation environments.

Expression of *CENH3* genes in rye and *Secalotriticum* hybrid genomes

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In the rye genome, paralogous genes were identified that encode two forms of centromeric histone proteins, αCENH3 and βCENH3, which define the region of kinetochore formation on chromosomes. We found that these genes are part of the 218 kb CENH3 locus. The presence of two paralogous genes raises questions about their possible subfunctionalization. We investigated the expression of genes at different stages of individual development of rye varieties and rye-wheat amphidiploid hybrids. These hybrids were obtained by crossing tetraploid rye (/RRRR, 2n= 4x=28) with hexaploid triticale (/RRAABB, 2n=6x=42) and were named secalotriticum (Secalotriticum, /RRAABB, 2n=6x=42). The RT-qPCR method revealed that the transcription levels of *CENH3* genes significantly decrease in vegetative tissues, and increase many times in reproductive tissues (2-3 times higher in anthers than in coleoptiles). The transcription level of *αCENH3* gene exceeds that of the evolutionarily younger *βCENH3*. The fact that *CENH3* genes activity in sekalotriticum remains at the same level with parental forms indicates the ability of centromeres to adapt to a new genetic environment in allopolyploid hybrids. Immunostaining of "Imperial" rye chromosomes and DNA fibers with specific anti-αCENH3 and anti-βCENH3 antibodies showed that αCENH3 and βCENH3 are co-localized on centromeres in the interphase nucleus and in metaphases of mitosis and division I of meiosis. Various patterns of nucleosomal organization were found on DNA fibers: clusters of signals with αCENH3 alternating with signals of βCENH3 and separated by clusters with canonical histone H3. The presented results indicate the most common pattern of duplicated gene expression, namely, a quantitative increase in the production of the expressed CENH3 protein.

Genetic and selection approaches to assessing the content and structure of winter rye arabinoxylans

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Arabinoxylans (AX) are pentose-containing carbohydrate biopolymers and secondary metabolites of the cereals cell walls. They are the special technological significance in the processing and forming the quality of the final product during grain grinding, baking, feeding and brewing, due to their high viscosity and water holding properties. The quality composition and pentosans content is a differentiating indicator that determines the direction of rye grain diversification. Creating varieties of diverse use is a priority task of winter rye breeding.

The range of methodological approaches and characteristics that allow the most objective and cost-effective assessment of breeding material on the content of pentosans has been expanded. Water extracts of whole meal flour of winter rye cultivars and lines were characterized on their rheological properties, arabinoxylan content, monosaccharide composition, and molecular weight distribution of carbohydrates. The higher amount of AX corresponded to the higher viscosity of the solution and vice versa. The mechanisms of formation of high and low arabinoxylan content in rye grain are proposed. Contrasting selection criteria should be applied depending on the end use in the quality rye breeding program.

In low-pentosans lines, a significant correlation was found between the water extract viscosity of rye meal and the content of the water-soluble AX. In high-pentosans varieties, polysaccharides are super massive molecules involved in the formation of high-viscosity solutions.

Generation of such diversity is complicated by two hundred years of artificial rye selection in favour of high-arabinoxylan content genotypes. Low-arabinoxylan content forms should be created purposively. This, in turn, requires the understanding of the mechanisms, which determine the level of arabinoxylans in rye grain.

Identification and characterization of putative brown rust resistance genes in rye (*Secale cereale* L.)

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One of the most important rye disease in Europe is brown rust (BR), caused by *Puccinia recondita* f.sp. *secalis* (Roberge ex Desmaz), (*Prs*). In other cereals, like bread wheat, the resistance to this pathogen can be acquired by the presence of specific resistance genes. To date, no BR resistance genes ware isolated or characterized in rye at the molecular level. The aim of this study was to find resistance gene candidates in three rye inbred lines bred in our Department. In the first step of this study, an *in silico* identification and characterization of BR resistance gene candidates were conducted. For this purpose, the nucleotide sequence of known genes, which confer BR resistance in *Triticum aestivum* and *Hordeum vulgare*, was used to search orthologues of these genes in recently published rye genome (Rabanus-Wallace et al. 2021). The gained sequences were bioinformatically analysed in various ways e.g. if they have an intact domains, conserved motifs and functional open reading frame. The most prospective gene candidates were selected for *in vitro* validation. The obtained PCR products were sequenced and analysed. The results suggest that tested rye lines possess orthologues at least a few of known BR resistance genes: *Lr1*, *Lr10, Lr21, Lr22a* and *Lr67*. Moreover, the structure of this genes revealed, that some of them may be functional in response to *Prs* infection.

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Does the gene *ScLr1* confer the resistance to brown rust?

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One of the most troublesome rye (*Secale cereale* L.) disease is brown rust (BR), caused by the biotrophic fungus *Puccinia recondita* f.sp. *secalis* (*Prs*). Up to date, 17 *Pr* genes related to the BR resistance in rye have been described. Most of them confer resistance at the seedling stage. Nevertheless, none of these genes have been characterized by molecular methods. The aim of the presented work was to identify an orthologue of wheat *Lr1* gene in a newly published genome of rye line Lo7. Moreover, the expression level of this gene in *Prs* and mock treated plants of three Polish rye inbred lines: L318, D33 and D39 was studied. Out of a dozen possible wheat orthologues, one gene, with the greatest sequence identity and coverage, was selected and named *ScLr1*. The gene *ScLr1* of line Lo7 has a typical R gene structure with NBS domain. The analysis of *ScLr1* gene in three other inbred lines showed almost complete sequence and structural compatibility between the lines Lo7 and D39. For lines D33 and L318, only short fragments of high homology (covering NBS domain) were found. In all three inbred lines, the level of *ScLr1* transcripts increased significantly after the first 17 hours after infection with partially compatible *Prs* isolate. Based on these results, we suggest that the analyzed variant of wheat *Lr1* orthologue can play an important role in early reaction to BR, at least triggered by a partially compatible *Prs* isolate.

Ways of using rye to increase resilience and bring diversity to the field and the table

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The need for food remains the unavoidable necessity of humans. The way we respond to this need determines the relationship between us and the environment. There is now widespread consensus that converting to regenerative farming practices enables us not only to farm with less harm to the environment, but also to mitigate some of the damage that has already been done. Regenerative farming means taking care of the soil through using cover crops, lessening the amount of inputs (fertilizers, herbicides and pesticides) used and increasing the diversity of the crops grown. Rye is a valuable addition for all these reasons. It has an expansive root system, weed-suppressing qualities, tolerance for cold temperatures, relatively low water requirements and resistance to diseases. Rye could be used more widely as a cover crop, but also grown in its own right as a cash crop. Its potential for intercropping has not been explored much. This poster will discuss the advantages and challenges of growing together wheat and rye, a mix historically known as 'maslin', in terms of the agronomical, culinary, and nutritional aspects.

INDEX

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