



# DIGITAL BREEDING

## Book of Abstracts

February 11-13, 2020 | Tulln – Austria

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Internationales Symposium der  
Gesellschaft für Pflanzenzüchtung e.V. (GPZ)

International Symposium of the  
Society for Plant Breeding e.V. (GPZ)



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## DIGITAL BREEDING

Internationales Symposium der Gesellschaft für Pflanzenzüchtung (GPZ) gemeinsam mit der  
Vereinigung der Pflanzenzüchter, Saatgutproduzenten und Saatgutkaufleute Österreichs

International Symposium of the Society for Plant Breeding e.V. (GPZ) in cooperation with  
Saatgut Austria

February 11-13, 2020 | Tulln – Austria

<https://gpz2020.boku.ac.at/>

hosted by:

**University of Natural Resources and Life Sciences, Vienna**

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Dear colleagues,

over the past decades, Vienna has become a European beacon of fundamental and applied plant research. Therefore, I am pleased that the GPZ Symposium on Digital Breeding will be organized and hosted by our colleagues at the University of Natural Resources and Life Sciences (BOKU).

The Society for Plant Breeding e.V. (GPZ) aims at advancing fundamental and applied research into plant breeding and strengthening the interaction between plant breeders and academia. GPZ was founded in Göttingen in 1991 and has more than 850 members at present. Every two years, the society organizes a scientific symposium at different locations covering topical issues of plant breeding. This year's conference sets a historic landmark because it is the first general symposium held outside Germany.

Agriculture faces humongous challenges in terms of food security, sustainability, biodiversity and global change. We are not going to solve any of these issues at the national level. The quest for innovation in plant breeding is a global mission. Against this backdrop, this conference will be a platform to foster scientific collaboration in the emerging field of Digital Breeding at the European level.

Our thanks go to the local organizers, especially to Prof. Dr. Hermann Bürstmayr and his team, who have put together an exciting program. It features outstanding keynote presenters from the international arena along with a lineup of young scientists from a wide range of institutes.

Again, a warm welcome to Vienna and best wishes for a rewarding conference.

A handwritten signature in black ink, reading "Andreas Graner". The signature is written in a cursive, flowing style.

Andreas Graner  
President of the GPZ

Dear colleagues and participants,

with great delight we took over the mission to host the 2020 General Plant Breeding Conference under the auspices of the Society for Plant Breeding e.V. (GPZ) at the Campus Tulln of the University of Natural Resources and Life Sciences Vienna (BOKU).

The general motto of the conference DIGITAL BREEDING seems to be a perfect choice as we received a total of 114 contributions, which underlines the spiritedness and engagement of the plant breeding community. We especially thank all of you who have responded to our invitation to submit abstracts of your latest research results to discuss these here with a broader audience. GPZ symposia are always highly inspiring as we can enjoy an interesting mix of contributions covering a broad range of crops and topics while at the same time giving both early stage and senior scientists the opportunity to present and critically discuss their recent work. A notable proportion of the scientific presentations comes thus from early stage researchers, some of whom get the chance to present their results for the first time at a high-level scientific meeting, and I personally always enjoy the enthusiasm and devotion of the younger generation. An outstanding feature of GPZ symposia is that its attendees represent numerous sectors, such as applied breeding, academia, administration or NGOs. It is you who make this symposium a fascinating and inspiring one!

I sincerely acknowledge all colleagues who served in the scientific advisory board for their recommendations and guidance in designing the program, and particularly for their active contributions as abstract reviewers for the numerous oral and poster contributions.

At the same time, I thank the local organizing committee and our student helpers for their active role in the planning and implementation of this conference. My particular thanks deserve Mrs. Suanne Weber, our conference secretary, whom many of you had contact with. Susanne's support and enthusiasm were the cornerstones when managing the conference preparations in a smooth and successful manner.

Lastly, without sponsoring and industry support we would not be able to realize this symposium. Therefore, I express my sincere appreciation to all sponsors, supporters and exhibitors.



Hermann Buerstmayr  
and all members of the local organizing committee

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Oral 1 – Keynote Lecture

## **Past experiences and future directions for genomic selection in animal breeding**

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Genomic prediction aims to predict the genetic values or phenotypes of individuals from DNA data using panels of Single Nucleotide Polymorphisms (SNPs) that cover the entire genome densely. Hence, all sites that affect a trait are expected to be in linkage disequilibrium with one or more of these SNPs, i.e. their genotype can be predicted from the SNPs. A statistical prediction model is developed using a training population of genotyped and phenotyped individuals. The prediction model fits all SNPs simultaneously, assuming the SNPs are random effects coming from a prior distribution. Alternative choices for the prior distribution are: the Normal distribution (resulting in GBLUP), the t-distribution (resulting in BayesA), a mixture distribution of a spike at zero and the t-distribution (resulting in BayesB), a mixture of a spike at zero and a Normal distribution (resulting in BayesC), and a mixture of a spike at zero and several Normal distributions (resulting in BayesR, which approximates BayesB using Normal distributions). The accuracy of the prediction depends on many variables: training population size, trait heritability, effective size of the population (affecting the linkage disequilibrium between sites), marker density (affecting the fraction of the genetic variance explained by the markers), genome size, number of genes and distribution of effects in combination with the statistical method of analysis. If the gene density is large across the genome, there are genes everywhere and the GBLUP method yields the highest accuracy. Generally, the Bayesian methods can more accurately describe the trait genetic architecture, and thus yield somewhat more accurate predictions. However, their improvement in accuracy is small, because trait architecture has been found to be very complex, in many cases involving thousands of sites affecting the trait.

Oral 2 – Invited Lecture

## **Using advanced digital phenotyping to identify novel breeding targets: stories about controlled environmental fluctuations, multi-trait dynamics and acclimation capacity**

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In their natural environment, plants continuously have to cope with short-term changes in environmental conditions such as light or temperature. It is known that, compared to constant environments, fluctuating conditions negatively impact plant performance and biomass production due to permanent acclimation. Predictions of future climate scenarios foresee even more extreme fluctuations. Thus, a detailed understanding of acclimation capacity, defined as the ability to lower the fluctuation-induced reduction in plant performance, is of utmost importance for securing yield stability. Furthermore, accessions with higher acclimation capacity will enable to identify novel breeding targets for the future.

We use high-throughput plant phenotyping facilities to monitor architectural and physiological changes in response to changing conditions (Junker et al. 2015, Tschiersch et al. 2017). Thereby we assessed growth dynamics and photosynthetic performance during early seedling establishment and vegetative development of about 300 IPK Genebank maize accessions under constant and changing temperature regimes (benchmarked against commercial hybrids). We were able to identify candidate accessions with superior performance in cold-tolerance and early photosynthetic efficiency, whereas the latter was found to discriminate hybrids and GB accessions through a machine learning-based approach. We identified variation in photosynthetic acclimation to switches in light intensity in both maize and *Arabidopsis*, which is currently subject of an association study in a F2 cross population and in haplotype groups of natural accessions discriminated by an environment-associated SNP in a potential target gene. We furthermore identified novel drought-acclimation-related genes of interest through an ML-based multi-omics analysis integrating phenomics and transcriptomics.

The implementation of a novel and unique plant growth and phenotyping facility at IPK will allow to extend these studies in a controlled and reproducible field-similar setup to enable the dissection of complex traits and to gain a deeper understanding of dynamic acclimation-related processes, especially in the context of early vigor.

Oral 3

**Phenotyping for crop improvement – technologies – access – knowledge**Roland Pieruschka<sup>1</sup>, Ulrich Schurr<sup>1</sup><sup>1</sup>Forschungszentrum Jülich Ulrich Schurr    [u.schurr@fz-juelich.de](mailto:u.schurr@fz-juelich.de)

Quantitative analysis of structure and function of plants has become the major bottleneck for many applications ranging from functional genomics to (pre-)breeding, breeding and analysis of biodiversity, and plant phenotyping has become an essential tool to address this gap. In recent years, significant interdisciplinary approaches have been started to establish plant phenotyping facilities to quantify the dynamics and the heterogeneity of plant structure and function, as well as of environmental cues. In this presentation, we will explain recent results to establish a plant phenotyping infrastructure to study the application and relevance of phenotyping technologies at various scales from the lab to the field in direct experimental approaches and from meta-analysis. The integration of different scales is also a central element of EMPHASIS: European Infrastructure for multi-scale Plant Phenomics and Simulation for food security in a changing climate, which is developing on the basis of the portfolio of existing national plant phenotyping centers in Europe. Here we will discuss the recent developments since EMPHASIS has been established as a ESFRI project. Additionally, the EU funded project EPPN2020 currently provides access to more than 31 phenotyping facilities, enabling high quality research with a number of best case examples of applications of plant phenotyping installations. Finally, we will introduce the latest activities of IPPN e.V., which is a networking platform linking the plant phenotyping centers and enabling close interaction across the globe.



Oral 4

## Can phenotypic marker-assisted selection for drought tolerance replace stress-trials in potato?

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Maintenance of yield stability requires efficient selection for drought tolerance, as climate models predict an increased likelihood of droughts. Yield-based selection for drought tolerance requires time-consuming drought trials. Marker-assisted selection (MAS) could accelerate tolerance breeding. An important crop for drought tolerance improvement is *Solanum tuberosum* ssp. *tuberosum*, which combines high water-use efficiency with low drought tolerance. The significant tolerance variation in tetraploid European cultivars provides a genetic basis for breeding. For this gene pool, we developed a tolerance prediction model based on leaf metabolite and transcript levels. We compared the performance of a selection based on this omics marker model to a classic yield-based selection. Furthermore, we tested the predictive value of phenotypic markers gained from laser scanner imaging and thermometry.



From a population of 200 lines segregating for drought tolerance, a classic subpopulation was selected for superior tolerance based on tuber starch yield data from 4 stress trials. Metabolite and transcript levels in leaf samples from these trials were used to predict drought tolerance by the random-forest model and select a tolerant MAS subpopulation. To compare their performance, these populations were characterized for yield and drought tolerance in ten multi-environment trials. The micro-meteorological characterization of the test environments indicated a good representation of relevant drought scenarios in agro-environments.

Yield data indicated that lines with drought tolerance above the mid-parent mean and yield potentials similar to released cultivars were identified by both selection procedures. However, the MAS procedure is faster than the selection based on yield trials. Among the parameters gained from automatic phenotyping, pre-dawn leaf angle and canopy temperature were the most promising drought tolerance predictors.

### Acknowledgment

This work was funded by the FNR/BMEL, GFPI and GTZ.

Oral 5

**Field phenotyping identifies the architectural and physiological functions determining canopy light interception and light use efficiency in winter wheat**Carolin Lichthardt<sup>1</sup>, Tsu-Wei Chen<sup>1</sup>, Andreas Stahl<sup>2</sup>, Hartmut Stützel<sup>1</sup><sup>1</sup>Leibniz University Hannover, Institute of Horticultural Production; <sup>2</sup>Justus Liebig University Giessen, Department of Plant Breeding Tsu-Wei Chen     chen@gem.uni-hannover.de



Canopy light interception efficiency (LIE) and light use efficiency (LUE) determine the biomass production and are important traits related to grain yield. To achieve further improvement of the modern high yielding winter wheat cultivars, it is important to discover the genetic variation in LIE and LUE and to understand the architectural and physiological functions of the whole canopy determining them. Using 220 winter wheat cultivars grown in field experiments conducted in three consecutive years (2015-2017), we demonstrate a mathematical framework, which estimates the architectural and physiological canopy functions by a simple field phenotyping protocol. Canopy traits, e.g. leaf area index, relative light interception, relative leaf chlorophyll content and canopy greenness were measured every week non-destructively from vegetative to grain filling stages. Using these traits, LIE, LUE, green canopy duration, green leaf area integral, canopy chlorophyll content, light extinction coefficient and nitrogen re-allocation rate were derived. Broad-sense heritability of all measured and derived traits ranged from 7-66%, with 22-78% phenotypic variation observed among all cultivars. LIE and LUE were not correlated and had the highest explanatory power for grain yield (30% and 64%, respectively). Using a structural equation modelling approach, green canopy duration and maximal leaf area index were identified as the canopy traits explaining 78% of the variation in LIE, and the relative leaf chlorophyll content explained 51% of LUE. Since all parameters in our field phenotyping protocol can be estimated by remote sensing using drones, our mathematical framework provides a new avenue for large scale field phenotyping in the era of digital breeding.

Oral 6

## Using NMR metabolomics in breeding for malt quality in spring barley

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Selective breeding in agriculture increases the genetic potential of plants and animals to show high performance for phenotypic traits by selecting the best selection candidates genetically as parents of the next generation. This genetic gain is a direct result of the accuracy by which we can predict the breeding values of selection candidates in each generation. The more accurate the breeding values, the higher the probability that we will select the best candidates, and the higher the genetic gain. Unfortunately, the accuracies of breeding values for malt quality in barley are somewhat lower than theoretically possible. They range between 30-50% for most traits, which highlights that we are only realizing a small proportion of the potential for genetic gain. The main reason is that the information currently used to predict breeding values – phenotypic, pedigree, and genomic data – does not tell us enough about the genetic potential of individuals. Other sources of information are clearly needed. Therefore, if we are to realize more genetic gain in barley, we need to uncover new sources of information that enable us to generate more accurate breeding values. An exciting new source of information is nuclear magnetic resonance metabolomics or NMR metabolomics. NMR metabolomics measures the abundance of all metabolites in a sample from an individual. These metabolite abundances (whole-metabolomic data) are associated with the level of physiological activity in biological pathways that are initiated at the DNA and culminate in trait expression. The level of physiological activity is regulated by the genes that an individual has inherited from its parents and by cues from its environment. This link between metabolite abundances and inherited genes means that individuals with high performance for individual traits should display similar patterns of metabolite abundances across the metabolome with these patterns reflecting common genes and environmental cues. In this study, which included more than 2500 malt samples from more than 500 individual spring barley lines from the Nordic Seed breeding population, we show that whole-metabolomic data could be used to accurately predict malt quality phenotypes of individual lines and plots in spring barley. Correlations between predicted and observed phenotype were 0.78 for filtering speed, 0.51 for extraction yield, 0.85 for wort color, 0.82 for beta glucan, and 0.80 for viscosity. This demonstrated that patterns of metabolite abundances did reflect common genes and environmental cues.

Oral 7

**Analysis of drought tolerance in perennial ryegrass (*Lolium perenne* L.) with methods of metabolite profiling and systemic metabolite markers**Johannes Wittmann<sup>1</sup>, Diana Drettwan<sup>1</sup>, Peter Westermeier<sup>2</sup>, Evelin Willner<sup>3</sup>, Stephan Hartmann<sup>2</sup>, Roland Geyer<sup>1</sup>

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The year 2018 was characterised by extensive drought events in Europe with large impact on yields even in intensive high yielding grassland regions. Perennial ryegrass (*Lolium perenne* L.), as one of the most important forage grass species and in many cases the dominant species in intensive swards, is likely to be particularly affected by such weather extremes. Current varieties have a limited tolerance to temporary drought, thus grassland yield is affected. The aim of the project was to efficiently improve tolerance of perennial ryegrass to temporary drought through enhanced recovery after drought using innovative selection methods. One of these methods, presented here, is metabolite profiling by nuclear magnetic resonance (NMR) spectroscopy. Lifespın developed an NMR-based method perfectly suited for analytical accompaniment of breeding programs – providing both quantitative metabolite data in sufficient quality and deepness and affordability for breeders. NMR is quantitative by nature, has a universal and unbiased detection principle and a broad dynamic range, and these advantages are enhanced with high-throughput amenability, reproducibility and easy to use handling.

The presented DRYeGRASS-System combines optimized sample preparation processes, automated NMR-measurement and metabolite profiling. The method enables the analysis of more than 100 samples per day, with walk-away capacity of even more than 480 samples. Resulting spectra are automatically processed and cover a dynamic range of more than six orders of magnitude, providing comprehensive metabolite profiles. The profiling software screens more than 200 metabolites and finally quantifies positively identified substances. Furthermore, the resulting metabolite profiles of >1200 single plants out of eight segregating, connected crossing populations were correlated with phenotypic data for drought tolerance obtained in a randomized two-location rain-out shelter experiment. The samples included five randomly distributed standard genotypes representing the population parents. During vegetation periods of 2017 and 2018, watering was withheld twice a year for up to four weeks for inducing drought stress and after cutting of the plants, a well-watered period of 4-6 weeks followed until the next cutting date. Sampling for the metabolite profiling was performed 10 days after first cut in both years, thus before the first stress was induced. First results and putative biomarker/metabolite profiles for drought tolerance will be presented. Classification performance of ~70% accuracy allows for drought tolerance selection in an early stage and might result in significantly increased efficiency in perennial ryegrass breeding.

The research leading to these results received funding from the German Federal Ministry of Food and Agriculture (BLE Innovationsförderung; FKZ: 2818209615, DRYeGRASS).

Oral 8

## Easy Breed - The flexible software solution for the entire breeding process

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Valid data and efficient workflows are very decisive for a successful development of new varieties. New promising methods to access your varieties directly in the field or in the lab will generate more and more data. Keep up with innovative analysis and data interpretation strategies as an advantage in the competition for new varieties.

With **Easy Breed**, the Wintersteiger AG would like to offer you a software solution to support and automate your current breeding process without restricting your freedom.

**Simplify Breeding Process - Easy Breed** significantly backs your breeding processes, e.g. creation of crosses and offsprings, designing of field experiments, generation of working lists, importing machine-based and manually collected data, statistical evaluation of data, selection of genotypes, and generation of result reports. Data from your whole breeding cycle, like scoring, yield and lab data including meta-data, pedigrees, genomic data, weather and soil information are sustainably integrated and are meaningfully displayed. Data can be queried and consolidated in a user-friendly way and can easily be found in subsequent years.

**Open for Future - Easy Breed** is and wants more; it is flexible to make sure that data of future technologies are covered. It has open interfaces to make sure that newly developed analysis workflows can be quickly integrated, assessed and used by our partners. With Easy Breed, we like to offer an interface to make the exploitation of tools and methods and even data sets developed in scientific projects more efficient.

**Adaptable and User-Friendly - Easy Breed** can be installed quickly as a standalone or server version. Existing data from breeding programs can be easily imported and supplemented by publicly available data, e.g. from plant variety offices. User-specific configurations, adaptations of workflows and complete user-specific extensions can be implemented in Easy Breed within a few hours or days. The software is currently available in German and English

**Easy Breed** - let's make breeding more efficient.

Oral 9 – Invited Lecture

## **Establishing tools for a fast-track genetic improvement of the wild crop species *Crassocephalum crepidioides*, to realize its potential as a nutritious, leafy vegetable**

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The tetraploid *Crassocephalum crepidioides* and its diploid relative *C. rubens* are underutilized, traditional leafy vegetables and medicinal plants that are native to tropical Africa, but grow throughout tropical and sub-tropical regions of the world. In folk nomenclature, depending on the region, various common names exist; most refer to both species. In Africa, common names are ebolo (Nigeria), gbolo (Benin) or akogbo. English names include fireweed, thickhead, and redflower ragleaf. *C. crepidioides* and *C. rubens* belong to the family *Asteraceae*, are annuals that propagate rapidly, grow well even in marginal soils and are rich in vitamins, minerals and essential oils.

Despite their value as food sources in Western and Central Africa, ebolo is not regularly cultivated, but is still mainly harvested from the wild and thus efforts are made to promote its domestication. To contribute, my group is investigating traits of relevance and generates tools and resources for a fast-track domestication of these orphan crop species. In cooperation with the African Orphan Crops Consortium, a sequencing and *de novo* assembly of the *C. rubens* genome was initiated. Moreover, regeneration methodology and transformation procedures are being established and a mutant collection is being developed. In addition, we are comparing traits of major relevance for the improvement of the plants between different African and Asian accessions using physiological studies and biochemical analyses. In this regard, importantly, we have obtained evidence that *Crassocephalum* species can accumulate large amounts of the highly toxic pyrrolizidine alkaloid (PA) Jacobine. PAs are present in approximately 3% of all flowering plants and frequently contaminate food and feed products. Results on the differences in PA accumulation depending on the genotype and the growth conditions applied will be presented and strategies that we plan to use for the targeted genetic removal of PAs, as a first essential step in the domestication of *Crassocephalum* species, will be discussed.

Oral 10

## **Mutations in SEED FATTY ACID REDUCER genes increase seed oil content in oilseed rape**

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Increasing seed oil content in oilseed rape is one of the main breeding goals. Seed oil content is a typical quantitative trait controlled by many genes. The final oil content in seeds is determined by the balance between both anabolic and catabolic pathways. It has been known that the seed oil content declines during seed maturity in oilseed rape. However, genes involved in oil degradation have not been deeply studied so far. We followed a novel strategy to increase seed oil content, not by increasing the transcriptional activity of lipid synthesis genes, but by knock-out of GDSL-type lipases termed 'SEED FATTY ACID REDUCERS', which are involved in oil degradation. We used targeted (CRISPR-Cas mediated) and random (EMS) mutagenesis to modify turnover rates of seed oil in winter rapeseed. We found significant increases in seed oil content after knocking out members of the two gene families without adverse effects on seed germination and seed vigor. Our results offer new perspectives for improving oil yield through molecular breeding. Moreover, we studied the inheritance of CRISPR-Cas mutations over 3 generations, which sheds light on the activity of Cas9 in primary transformants and their offspring.

Oral 11

**Hybrid speciation in *Brassica***

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Hybridization (where two species come together to form a new species) has played a major role in evolution and speciation, particularly in the flowering plants. Many crops grown today are hybrid species, including wheat, canola, sugarcane and cotton. However, exactly how hybrid species form is still a mystery in almost all genera. New hybrids face a number of challenges to establishment, the most serious of which may be regulating meiosis. In most newly-formed hybrids, two sets of chromosomes from each of the progenitor species will be present. These sets of chromosomes not only contain competing genomic information, but almost always share regions of genomic similarity that can hinder homologous chromosome pairing and segregation. Failure of meiosis to correctly segregate chromosomes belonging to different genomes usually results in loss of chromosomes and genomic information from one generation to the next, and subsequently loss of fertility and true-breeding in offspring. Hence, hybrids must regulate meiosis to establish as new species. But how? In the *Brassica* genus (cabbages, turnips, canola, mustards), cultivated species share combinations of the A, B, and C genomes: AA, BB, CC, AABB, AACC and BBCC genome-types all exist as established crops. We produced novel interspecific hybrid types through crosses between the AABB, AACC and BBCC species, and analysed resulting interspecific progeny over several generations using a combination of molecular cytogenetics and high-throughput molecular marker karyotyping approaches. Three new, meiotically stable hybrid types were produced from segregating progeny: one with an AABBCC genome complement, and two with a recombined A/C genome from crosses between the AABB and BBCC species. We identified both genetic variants of meiosis genes inherited from the progenitor species and similarity between subgenomes in the novel interspecific hybrids as major factors affecting the success of speciation events via interspecific hybridization events in the *Brassica* genus. Our results shed light on the mechanisms of repeated hybridization and speciation in this major crop genus, and point the way towards production of new crop types.



Oral 12 – Invited Lecture

## Unlocking the polyploid potential of wheat through genomics

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Several developments over the past 18 months have radically changed the way we work with polyploid wheat. Both hexaploid and tetraploid wheat now have whole genome sequences and reliable gene models. This has expanded beyond the single reference genome to multiple cultivars. We have developed *in silico* mutant resources with over 95% of genes with either a knockout or deleterious allele in both tetraploid and hexaploid wheat. We have a comprehensive gene expression atlas in wheat with over 1,000 RNA-Seq samples along with co-expression and transcription factor target networks. All this data is open-access and displayed at *EnsemblPlants*. Novel strategies have accelerated cloning of disease resistance and other genes. Using accelerated growth conditions (speed breeding), the community now routinely grows wheat in 10-week seed-to-seed cycles compared to the previous 16-20 weeks. All these developments have dramatically lowered the barriers to undertake biological research in polyploid wheat. For many purposes, wheat can now be treated (almost) like a model crop species. The next phase will be to start understanding the biological mechanisms underlying the most important traits in polyploid wheat and to design strategies to ensure this knowledge is quickly transferred to the field. We argue that given polyploidy, breeders have exploited only a fraction of the potential genetic variation in the wheat genome. The recent breakthroughs in wheat genomics now allow us to make a decisive effort towards exploiting this underutilised variation, thereby unleashing the full potential of the wheat genome.

Oral 13

## Characterization of a large panel of maize nested association mapping near-isogenic lines (NAM NILs)

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Over the past decade, nearly 100 genome wide association studies in maize inbred diversity panels and nested association mapping (NAM) populations have been published. These studies have implicated hundreds of quantitative trait loci (QTL) in the control of dozens of complex traits. Near-isogenic lines (NILs) have been used extensively to characterize, validate, and dissect candidate QTL in maize. However, the development of NILs is time-consuming and costly, as it requires genotyping and several generations of backcrossing and selfing. As such, NIL populations have been limited in size and introgression donor diversity in the public sector. Syngenta AG has recently made public a large panel of maize NILs derived from crosses between the 26 diverse inbred founders of the maize NAM population. We characterized 1,270 NILs containing introgressions from 18 diverse inbreds in a B73 background (NAM NILs) from the greater Syngenta panel. The NAM NILs were phenotyped for six quantitatively inherited traits that had been previously characterized with the maize NAM population: days to anthesis, ear and plant height, and resistance to the fungal foliar diseases gray leaf spot, northern leaf blight, and southern leaf blight. We used genotyping-by-sequencing to identify the physical positions and donors of the introgressions present in the NAM NILs. For all traits, phenotypic variation and heritability were relatively high. The majority of the NAM NILs each contained four or fewer introgressions. The introgressions present across the NAM NIL panel spanned the entire genome and demonstrated substantial allelic replication. We identified QTL associated with height and disease resistance and found donor allelic variation at all associated QTL. To date, this is the largest, most diverse publicly available panel of maize NILs to be phenotypically and genotypically characterized. The NAM NILs are an invaluable resource for the maize genetics community.

Oral 14

## Genome-wide association mapping of agronomically important traits in quinoa



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Quinoa (*Chenopodium quinoa* Willd.) is a traditional Andean crop that was domesticated around 5000-7000 years ago. Quinoa consumption has rapidly expanded in recent years due to its high nutritional value. However, breeding is still in its infancy. Predominantly, Quinoa is a short day crop. For cultivation in Northern Europe, it must be adapted to the long days through modification of flowering time. Therefore, we aimed to identify the genetic basis of flowering time regulation and agronomically important traits by genome-wide association study in quinoa. A diversity panel of 324 quinoa accessions from different geographical locations was grown in the field in Kiel. The accessions displayed large phenotypic variation with regard to flowering time and other agronomically important traits. All the accessions of the diversity panel were sequenced by a whole-genome re-sequencing approach. We identified SNPs based on the QQ74 genome V2 reference assembly. We obtained 3 million high-quality SNPs, which we used to identify significant associations between SNPs and important traits, such as flowering time, panicle length, plant height, saponin content, and seed yield. Presently, we are identifying candidate genes for flowering time. We observed rapid LD decay in the diversity panel indicating a weak genome-wide footprint of breeding and selection in the history of quinoa. We aim to use sequence variations to select promising accessions as crossing partners to breed new varieties well-adapted to European climate conditions. Our study provides resources for fast track genetic improvement of the underutilized pseudocereal quinoa. This will enable harnessing its undiscovered potential in sustainable agricultural production.

Oral 15

**QTL mapping for *Fusarium* head blight resistance in wheat: a review**Maria Buerstmayr<sup>1</sup>, Barbara Steiner<sup>1</sup>, Hermann Buerstmayr<sup>1</sup><sup>1</sup>Department of Agrobiotechnology, Institute of Biotechnology in Plant Production, University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Str. 20, 3430 Tulln, Austria Maria Buerstmayr  maria.buerstmayr@boku.ac.at

The extensive past and ongoing research on *Fusarium* disease is reflected by the vast number of primary and secondary literature that covers diverse aspects and components of breeding for FHB resistance.

This review summarizes results of QTL studies conducted from 2009 onwards. Most studies conducted either single floret inoculation (SFI) measuring resistance to fungal spreading within the spike (type 2) or spray/grain-spawn (SPI) inoculation reflecting resistance to fungal entry (type 1) and overall field resistance. Fourteen of a total of 58 screened mapping populations were evaluated using SFI and SPI in parallel; around twice as many QTL were detected after SPI (84) than after SFI (40) including 18 QTL that conferred both type 2 and type 1 resistance. Generally, under field conditions, inconstancy of QTL was rather the rule than the exception, and only highly effective QTL were detected under a range of environments. Comparing type 2 with overall field resistance indicates that field resistance is much more complex and regulated by combined effects of many QTL (average 6.6 QTL), while type 2 resistance is primarily regulated by few QTL (average 3.3 QTL). Field resistance is furthermore strongly impaired by plant morphology and the environment. Overlap with QTL for FHB resistance were found for 60%, 40% and 25% of the reported QTL for anther retention/extrusion, plant height and heading/flowering date, respectively. Assessing and integrating these traits in the analyses will allow a more meaningful interpretation of the QTL results.



More than 450 QTL have been published since the first FHB resistance QTL in wheat was reported in 1999 (Waldron et al. 1999; Buerstmayr et al. 2009). Considering this large number, very few have been deployed in breeding programs as: i) breeders are reluctant to use unadapted resistance donors ii) the quantitative genetic architecture with primarily small to medium effects QTL makes gene pyramiding difficult, iii) apart from *Fhb1*, solely linked markers are available. Developing diagnostic markers, in particular for large effect QTL, generating better adapted resistant germplasm, considering trait associations in breeding decisions, and designing skillful genomic selection approaches will certainly increase breeding efficiency. *Fusarium* resistance is a key component in integrated *Fusarium* management and contributes to sustainable disease control and toxin prevention right at the beginning of the production chain: on the farmer's field.

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Oral 16

**Genomic and epigenomic patterns in novel heterotic pools of winter rapeseed (*Brassica napus*)**Jenny HueyTyng Lee<sup>1</sup>, Amine Abbadi<sup>2</sup>, Rod Snowdon<sup>1</sup><sup>1</sup>Department of Plant Breeding, Justus Liebig University Giessen, Germany; <sup>2</sup>NPZ Innovation GmbH, Hohenlieth, 24363 Holtsee, Germany Jenny HueyTyng Lee     huey.t.lee@agrار.uni-giessen.de

Exploitation of hybrid vigour in crops is simplified by distinct heterotic pools and breeding methods that facilitate effective prediction and use of heterosis. In crops which have traditionally been bred as open-pollinated inbred line varieties, like oilseed rape/canola (*Brassica napus*), heterotic pools generally do not exist and systematic exploitation of heterosis is challenging. Using winter oilseed rape as a case study, we are investigating how genome-wide patterns of genomic and epigenomic variation may help distinguish and develop new heterotic pools. We sequenced two pools of 50 elite, winter type oilseed rape lines, and catalogued the genomic and epigenomic variants of each genotype. As expected from the breeding history, single nucleotide polymorphisms and methylation patterns were found to largely overlap between pools. However, variants unique to pools were detected at the genomic level, indicating strong potential for genomics-assisted separation of heterotic pools. By tracing these divergent variants throughout a breeding program in intercrossed pool offspring, we are generating a catalogue of genomic and epigenomic patterns which will serve as a basis for hybrid performance prediction once recombinants are successfully fixed in individual pools through genomics-assisted crossing designs. Our approach introduces a novel exploitation of heterotic patterns to enhance the breeding process, which bypasses the need for direct association of each variant to the trait performance.

Oral 17

**Exploring natural genetic variation in meiotic recombination rates in barley**

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Meiotic recombination and independent assortment of homologous chromosomes during meiosis generates novel allelic combinations upon which plant breeders can act. As such, the frequency and distribution of meiotic recombination events determines the amount of allelic combinations found in subsequent generations. Meiosis is a highly regulated cell division, and the recombination landscape is shaped by genetic, epigenetic, and environmental factors, as well as their interactions. Here, we analyse recombination landscapes of a large nested association mapping (NAM) population composed of 1,420 lines grouped into 25 families derived from an intraspecific cross between domesticated barley and 25 wild barleys (HEB-25, Maurer et al., 2015). We identify natural variation in the total number of crossovers per line with a maximum of a 1.7-fold difference between families. A genome-wide QTL scan identifies *homologous-pairing protein 2 (HOP2)* as a putative candidate gene contributing to variation in total crossover number. Using exome capture data of all lines, we identify a novel haplotype of *HOP2* not present in the domesticated parent 'Barke' or the reference genome cultivar 'Morex'. We present further ideas to decipher the functional significance of the wild barley *HOP2* variant and its use in plant breeding.

Oral 18

## A novel wild allele improves drought adaptation and yield sustainability in cultivated barley

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Drought events have become more erratic in recent years, causing serious crop losses. The accumulation of the metabolite proline is one of the most generalized responses of plants against drought stress. Here, we used the natural diversity of barley to dissect genetic regulation of proline accumulation and its role in drought adaptation. Genetic mapping revealed a major quantitative trait locus (QTL; *QPro.S42-1H*) for drought-inducible proline accumulation in a library of wild barley introgression lines. Subsequent fine mapping and positional cloning showed that *QPro.S42-1H* underlies a previously unknown pyrroline-5-carboxylate synthase (*P5cs1*) allele originated from the wild barley accession ISR42-8. The causative allelic variations were found in the *P5cs1* promoter putatively across the DNA binding motifs of the abscisic acid-responsive element binding transcription factors (ABF). We proved genetic variation of wild and cultivated *P5cs1* promoter alleles using transiently expressed promoter::reporter constructs in *Arabidopsis* mesophyll protoplasts. Notably, gene expression and  $\beta$ -glucuronidase (GUS) activity was significantly higher in the ISR42-8 promoter compared to Scarlett upon abscisic acid treatment. The activation of the ISR42-8 promoter was impaired in the protoplasts isolated from loss-of-function *abf* mutants, suggesting that the wild allele promoter was modulated by ABF transcription factors. To test the performance of the *P5cs1* wild allele, we evaluated multiple breeding traits, yield components and physiological parameters under control, drought and field conditions. Notably, an introgression line (S42IL-143) carrying the *P5cs1* wild allele at BC3 generation demonstrated improved physiological activity and photosynthetic yield compared to Scarlett under drought stress. Next, we established a near isogenic line (NIL-143, BC6 generation) carrying the wild barley allele in cultivar Scarlett background to test yield and sustainability traits under drought stress in field conditions. Interestingly, the NIL-143 and Scarlett showed no difference under an irrigated block, but NIL-143 revealed a remarkable increase in grain yield per plant (+34%), thousand grain weight (+7%) and grain number per ear (+18%) as compared to cultivar Scarlett under field drought conditions. These findings on a natural variant of *P5cs1* contribute to develop drought resilient cultivars in barley as well as to investigate its role in drought physiology in related crops like wheat via comparative genomics.

Oral 19

## Genetic interplay of yield, baking quality and resistance in the MAGIC winter wheat population WM-800

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The MAGIC-WHEAT WM-800 project pursues the goal to develop new winter wheat cultivars with improved agronomic traits concerning yield, quality, pathogen resistance and nutrient efficiency. The multiparental winter wheat population WM-800 (Sannemann et al. 2018) is based on an eight-way cross of modern German varieties. For a better understanding of yield and quality parameters depending on nitrogen availability, the WM-800 population was cultivated under two contrasting nitrogen (N) levels and was investigated for the traits heading, anther extrusion, plant height, grain yield and the three yield components, thousand grain weight, grain number per ear and ears per square meter at four locations in Germany in 2017 and 2018. The traits protein and starch content as well as sedimentation value were detected to complement nitrogen related yield results with associated quality parameters. The WM-800 population was additionally grown without any plant protection products and was observed for three different plant diseases: stripe rust, leaf rust and *Fusarium* head blight. A genome wide association study (GWAS) was implemented as mixed linear model with 27,685 informative, physically positioned SNPs (RefSeq 1.0, IWGSC). Significant differences between the two nitrogen levels were found, thus GWAS resulted in nitrogen specific QTL for all yield and quality traits. These QTL can be implemented in breeding programs for nitrogen efficient genotypes with regard to a more stringent fertilizer application in the future. Furthermore, GWAS resulted in a set of resistance QTL for the investigated wheat diseases with scoring effects of up to -0.7 scoring units (FHB), -2.0 scoring units (leaf rust) and -1.6 scoring units (yellow rust). Combining positive allele effects for each breeding target within new wheat genotypes was one major aspect of the project. The ambivalent interplay of yield, baking quality and resistance is a well-known problem in winter wheat breeding. Remarkably, high recombination during population development enabled the detection of superior genotypes with favourable allelic effects among our WM-800 genotypes.

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Oral 20

### **Tissue specific global transcriptomes of barley meristems**

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The gross morphology of an organism can be traced to its early developmental events, particularly to the changes in genes controlling development. In plants, specification of various organ primordia, such as roots, leaves, and flowers, is majorly driven by the transcriptional regulation at the site of their specification. Hence, understanding the precise control of organ specification necessitates the need to dissect the transcriptional regulation at the site of organ initiation. The barley inflorescence called spike has a unique structure called triple spikelet (TS) [(one central (CS) and two lateral spikelets (LS))] along the inflorescence axis. The CSs are always fertile. The fertility of LSs at the TS distinguishes barley spikes into two- (sterile LSs) and six-rowed (LSs fertile). To understand the transcriptional landscape specifying the opposing fates of LSs and CSs, we precisely isolated immature LS and CS organs in the two-rowed cv. Bowman by applying laser-capture microdissection across seven spike developmental stages and subjected these samples for RNA-seq analysis. Besides, we also analyzed apical inflorescence meristem, spike pro-vascular tissue, apical root, and basal leaf meristems. Our analysis of differentially regulated genes between CS and LS tissues revealed the involvement of known and unknown regulators of LS fate and development. By using mutational and phenotypic analyses of three of the novel genes, we validated their differential transcriptional regulation between CS and LS. In summary, we have developed a high-resolution tissue-specific transcriptome atlas of meristems from developing barley spikes, illuminating the precise regulation of spike development.

Oral 21

## **Landscape genomics identifies the genetic architecture of soybean environmental adaptation and genetic resources suitable for Central European soybean breeding**

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Landscape genomics primarily focuses on the detection of adaptive genetic variation in natural populations that results from local adaptation to natural habitats. The same methodology can be applied to crop populations to identify the genetic architecture of region-specific environmental adaptation or to even detect (novel) candidate loci to improve crop adaptation.

We performed a set of analyses that were inspired by the landscape genomics rationale with the objective to study the genetic architecture of soybean adaptation to high-latitude cold regions. The investigation of population structure in a large germplasm collection ( $N \geq 17,000$ ; USDA Soybean Germplasm Collection<sup>1,2</sup>), aided by passport information, enabled us to restore the link between parts of the collection and the origin of material, resulting in a dataset of soybean accessions representative of major Chinese germplasm groups. Geographic information system databases<sup>3,4</sup> further permitted the characterization of the traditional growing environments of these groups and enabled the association of genetic<sup>5</sup> with environmental variation across agro-ecological gradients. By using BAYPASS<sup>6</sup> and QTCA<sup>7</sup> we identified loci that were highly genetically differentiated among populations and/or exhibited significant associations between local population allele frequencies and local environments. The inspection of the genome annotation within these differentiation and association signatures largely clarified their functional involvement in developmental processes such as flowering and maturity, as well as in abiotic stress responses. We are therefore currently assessing the allelic states of these genomic regions in modern North American varieties and in European varieties to evaluate the potential of Chinese germplasm to improve abiotic adaptation in modern material intended for high-latitude and cold growing environments. This work may identify beneficial variation in genetic resources that is not represented in modern germplasm and may reveal promising allele stacking strategies.

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Oral 22

**Association studies in roses reveal robust markers for flower traits**Dietmar F. Schulz<sup>1</sup>, Marcus Linde<sup>1</sup>, Thomas Debener<sup>1</sup><sup>1</sup>Institute of Plant Genetics, Molecular Plant Breeding, Leibniz University of Hannover, Hannover, Germany Dietmar F. Schulz     schulz@genetik.uni-hannover.de

Floral traits are the most important characteristics that determine the ornamental value of cultivated roses. A number of studies have been conducted on qualitative and quantitative factors influencing floral traits, but almost all of these studies were based on biparental populations. Here, we present data on markers generated through an association study in a set of 96 diverse rose genotypes for flower petal number and the verification of a marker in a set of independent populations. For marker analysis, we used a recently designed Axiom SNP chip comprising 68,893 SNPs with additionally 281 SSRs, 400 AFLPs and 246 markers derived from candidate genes. The mapping of markers significantly associated with petal number, scent emission and petal size and revealed clusters of associated markers indicating genomic regions associated with the traits. One of these genomic regions on chromosome 3 is located in vicinity of the DOUBLE FLOWER locus, where a dominant gene controls simple versus double flower phenotypes. Some of the associated markers show additive gene action over all five allele dosages in the tetraploid rose population. Genetic markers were developed based on the KASP technology, which can be considered as beneficial for marker-assisted selection in commercial breeding programmes in the future. Some of the markers with considerable effect sizes could be validated in a set of 250-300 independent genotypes. These markers for petal number are also starting points for functional genomic studies to identify the causal factors for the observed phenotypes.

Keywords: GWAS, SNP, KASP, MADS-box genes, marker-assisted breeding, petal number

Oral 23

**Widespread gene-scale structural variants revealed by long-range sequencing**

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There is increasing evidence that genome structural variation (SV) contributes strongly to trait variation in eukaryotic species. However, the impact of SV in complex, highly-duplicated plant genomes is difficult to assess without *de novo* genome assembly, due to an inability to effectively distinguish and assay small-scale SV events using second-generation sequencing technologies such as Illumina sequencing. Third-generation long-read sequencing technologies now make it possible to precisely detect small scale SV in a range of 30 to 1000 base-pairs. By analyzing medium coverage long-read sequencing data originating from 12 diverse *Brassica napus* genotypes, we were able to identify widespread, genome-wide, intragenic SV events, many of which were discovered in genes contributing to eco-geographical adaptation and other agronomically important traits. Our results suggest that revisiting complex plant genomes using long-read sequencing can reveal unexpected levels of functional gene variation, with major implications for trait regulation and crop improvement.

Oral 24

**Analysis of subgenome structure and evolution in allopolyploid plants**Matteo Schiavinato<sup>1</sup>, Alexandrina Bodrug<sup>1</sup>, Marina Marcet-Houben<sup>2,3</sup>, Toni Gabaldón<sup>2,3,4</sup>, Juliane C. Dohm<sup>1</sup>, Heinz Himmelbauer<sup>1</sup>

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Many crop plants are allopolyploid species, i.e. they arose by interspecific hybridisation. Thus, the genomes of these crops contain subgenomes, which were inherited from parental progenitors that were interfertile despite being distinct species. Examples of such crops with already assembled genomes include peanut, oilseed rape, tobacco, cotton, wheat, and quinoa. In the context of plant breeding, knowledge about the parental origin of a particular gene could be of interest, for instance, for choosing wild relatives for crop improvement. Distinguishing subgenomes is therefore an important task. The sequence identity between the subgenomes together with their evolution through time complicates their analysis. Two different ways can be used to distinguish subgenomes. Firstly, genomic sequencing data generated from the parental species or their living relatives are employed for subgenome differentiation by read mapping. Secondly, phylome analysis is conducted in cases where subgenomes are at an advanced stage of intermixing. We describe the two approaches using representative plant genomes and discuss the results. Importantly, hybrids may be at different stages of their genome evolution towards regaining a diploid state, which does not necessarily correlate with their age.

Oral 25 – Invited Lecture

## **Reverse genomic prediction: identifying important traits in breeding populations**

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Genomic prediction allows breeders to make selections based on genetic information, even without knowledge of the causal loci for a trait. Advantages conferred by genomic prediction are most pronounced for polygenic traits, because genetic mapping requires a population size that is inversely proportional to the effect of a given locus. Likewise, selection mapping, or identifying loci that have previously been under selection, is plagued by the same challenge: the ability to detect a selected locus depends on population size. This challenge can be even harder to overcome for selection mapping, since small population sizes in the past result in elevated genetic drift and restrict the ability to identify loci in the present. However, since selection on polygenic traits impacts allele frequencies at loci dispersed genome-wide, it leaves behind a whole-genome signal of selection. We have developed a method to test for this form of previous selection that is analogous to genomic prediction's use of all markers to drive future selections. Our test is based on the correlation between the allele frequency change of every genotyped locus and the estimated effect size of alleles at each locus. Unlike genetic mapping, our test is increasingly powerful for increasingly polygenic traits. Results from maize, wheat, brassicas, and chickens demonstrate that the method can powerfully detect selection on known candidate traits, and has the potential to identify additional target traits for continued population improvement.

Oral 26

## 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 other agronomic traits 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 in 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 ( $=0.72$ ) and highly variable among the spectrum. Correlations between DMY and single bands were generally significant ( $p < 0.05$ ) but low ( $\leq 0.29$ ). 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.



Oral 27

**The relevance of dominance to genomic selection in clonal breeding programs**Christian Werner<sup>1</sup>, Chris Gaynor<sup>1</sup>, Gregor Gorjanc<sup>1</sup>, Daniel Sargent<sup>1</sup>, Alessandra Lillo<sup>1</sup>, John Hickey<sup>1</sup><sup>1</sup>The Roslin Institute, University of Edinburgh Christian Werner     christian.werner@roslin.ed.ac.uk

Many major food crops, including nearly all types of fruit and all important root and tuber crops, as well as a wide range of forage crops and forest trees, are clonally propagated. Clonal breeding programs typically start with a crossing step, followed by multiple years of phenotypic mass selection or recurrent selection of clonally propagated individuals. This makes the development of new cultivars both time- and resource-expensive. Despite this, the general structure of conventional clonal breeding programs has barely changed over the last few decades. Genomic selection (GS) has great potential to substantially improve the efficiency of breeding for quantitative traits in clonally propagated plant species. However, evaluating the cost efficiency of different GS strategies requires measuring breeding program performance over a long time period, which is cost-prohibitive. We used stochastic simulations to evaluate different strategies to cost-effectively implement GS in breeding programs for clonally propagated plant species that exhibit diploid(-like) recombination during meiosis, such as a strawberry and cassava. Simulations were run over 20 years of breeding to compare two breeding programs with GS to a conventional clonal breeding program using phenotypic selection. Furthermore, two selection strategies for the identification of new crossing parents were compared, including the selection of parents based on i) their genomic estimated breeding value (GEBV), and ii) the predicted performance of each parental cross. We hypothesise that dominance may have a strong impact on the efficiency of breeding programs with GS. All individuals were heterozygous, and different dominance degrees were simulated to examine the impact of non-additive genetic effects on breeding program performance. Under all dominance degrees, the GS breeding programs generated more genetic gain over time than the conventional breeding program when prediction of cross performance was used to select new parental combinations. However, selection of new crossing parents using the GEBV promoted inbreeding depression, which adversely affected genetic gain under moderate to high dominance degrees. Our findings lay the foundation for the successful implementation of GS in practical breeding programs and the consequential investigation of more complex research questions, such as GS for multiple traits and the utilisation of GS in clonally propagated plant species with a polyploid genome structure.



Oral 28

**Ten years of genomic selection in an applied wheat breeding program – from expectations to experience**Sebastian Michel<sup>1</sup>, Franziska Löschenberger<sup>2</sup>, Christian Ametz<sup>2</sup>, Hermann Buerstmayr<sup>1</sup><sup>1</sup>University of Natural Resources and Life Sciences, Vienna; <sup>2</sup>Saatzucht Donau GesmbH. & CoKG Sebastian Michel    sebastian.michel@boku.ac.at

The advent of cost-efficient genotyping techniques enabled the routine fingerprinting of breeding populations with thousands of genome-wide distributed markers. The efficient usage of these fingerprints to genomically predict breeding values of early and advanced generation breeding material has been one of the major plant breeding topics in recent years. Using the example of an applied winter wheat breeding program, an overview about genomic selection for line variety development will be given, starting with the initial expectations when a pilot study was initiated in 2009-2012. Results and experiences from the first validation experiment in 2013-2014 will be reported as well as further prospects, progresses and challenges during its routine application in the program until 2019. Several case studies will be presented for this purpose, including the integration of genomic and phenotypic information for predicting grain yield and baking quality as well as the application of genomic index selection to combine these negatively correlated traits in bread wheat. Furthermore, some insight will be given into genomic selection for disease resistance on the example of *Fusarium* head blight, specifically addressing the frequently observed confounding effects with other agronomic traits like flowering biology and plant height. Challenges in the application of genomic selection with respect to strong genotype-by-environment interactions and an appropriate training population design will be discussed for biotic stresses in the light of stripe rust race dynamics and for abiotic stresses on the difficult and laborious-to-phenotype frost and drought tolerance. Lastly, a brief overview will be given on genomic cross prediction based on recent empirical and simulation studies, emphasizing the large importance of an appropriate diversity management when genomically planning crosses within a single program, as well as across different breeding programs in the framework of the breeders' exemption.

Oral 29



## Quantifying the contribution of epistasis to quantitative trait variation with Epistasis Mapping Populations

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In this study, epistatic interactions were quantified using a maize epistasis mapping population (EMP). A subset of twenty near-isogenic lines (NILs) were chosen from a publicly available set. The chosen NILs were crossed in a half-diallel scheme and backcrossed to their recurrent parent to develop a powerful resource for identifying pairwise epistatic interactions. Each pair of crossed NILs ("F1-NILs"), coupled with their backcrosses to B73 (backcrossed NILs; BC-NILs), forms a triplet that can be used to test for epistasis. In the triplet, two BC-NILs are heterozygous for a single introgression, and one F1-NIL is heterozygous for both introgressions. Together, the complete set of these triplets, which we have named an EMP, can powerfully test for epistasis between any two introgressions. By utilizing twenty NILs with relatively large introgressions, we developed a maize-based EMP that covers ~ 75% of the donor genome. The EMP mating design dramatically reduces the number of multiple testing corrections to account for, from millions or billions, to hundreds. Our maize EMP is based on the inbred lines B73 and Mo17. The EMP was planted in two trial years (2017 and 2019) to undergo field testing for epistatic interactions. We evaluated agronomic traits including flowering time and ear number, as well as ear traits based on a high-throughput image analysis pipeline that scored cob, ear and kernel size and shape. For all phenotypes measured, deviation from two-locus additivity was calculated. Preliminary results suggest that epistasis contributes substantially to phenotypic variation, particularly for fitness-associated traits.

Oral 30

**Omics-based prediction of hybrid performance in maize**Tobias Schrag<sup>1</sup>, Matthias Westhues<sup>1</sup>, Stefan Scholten<sup>2</sup>, Albrecht E. Melchinger<sup>1</sup><sup>1</sup>Universität Hohenheim; <sup>2</sup>Universität Göttingen Tobias Schrag  schrag@uni-hohenheim.de

Predicting the agronomic performance of single-crosses with high precision is crucial for selecting superior candidates in hybrid breeding. With advanced technologies, thousands of new parent lines can be generated in each breeding cycle. Therefore, millions of new hybrid combinations exist in principle, of which, however, only a small subset can be produced and phenotyped in multi-environment yield trials. Best linear unbiased prediction (BLUP) using pedigree data and whole-genome prediction using genomic data are well established prediction approaches, but limited in capturing epistasis and interactions within and among downstream biological levels such as transcriptome and metabolome. Information on factors which influence such biological levels is expected from mRNA and small RNA (sRNA) sequences as being involved in transcriptional, translational and post-translational processes. We combined genomic, transcriptomic (mRNA and sRNA) and metabolomic data of parent lines to evaluate the ability to predict the performance of untested hybrids for important agronomic traits in grain and silage maize. Considerable interaction with respect to predictive ability was observed between predictor and trait, where mRNA data were superior for yield and genomic data for dry matter content. Combining mRNA and genomic data as predictors resulted in high predictive abilities in most traits and combining other predictors improved prediction over that of the individual predictors alone. In summary, transcriptomics can complement genomics for hybrid prediction and improve selection of hybrid candidates.

## Comment

The talk refers to work published in Westhues et al. (2017, Theor Appl Genet) and Schrag et al. (2018, Genetics)

Poster 1

## **Increasing root biomass production in European winter wheat for improved drought stress tolerance and nitrogen use efficiency**

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A sufficient supply of nitrogen is one of the most essential factors in order to achieve high grain yields and quality of wheat, hence meeting the increasing food requirements of a growing world population. However, by applying nitrogen, a significant amount can escape from the cropping system by leaching into groundwater or by being transformed into volatile atmospheric emissions. Therefore, although nitrogen fertilisation will continue to play a major role in the production of high crop yields in the future, the negative effects of nitrogen fertilisation on ecosystems must be reduced. At the same time, water and nutrient uptake is strongly impacted by drought events, which are increasing in degree and frequency in almost all relevant wheat cropping regions. Hence, there is a great need for a strategic development of varieties that achieve higher yields under reduced fertilisation and erratically occurring drought phenomena. Recently, this has led to a growing interest into the understanding of the root system as the organ for water and nutrient uptake. However, knowledge about the development of wheat roots and their genetic control is insufficient. This is primarily due to difficulties in measuring of root functions compared to above-ground phenotyping. Reliable molecular markers for relevant root traits can enable breeders to overcome difficulties with underground phenotypic selection. In this study we create near-isogenic lines (NIL) of European elite winter varieties that carry introgressions of a major QTL which confers a larger root system. Backcross progenies are being subjected to foreground selection, using kompetitive allele specific PCR markers (KASP) for the desired QTL alleles conferring the larger root system. In order to evaluate the biggest genetic background of the elite variety, background selection is conducted after each backcrossing step using genome-wide markers. Furthermore, we are investigating the relevance of an increased root growth for enhanced water and N-uptake. This question will be clarified through (i) analysis of N-transfer with <sup>15</sup>N labelled fertilisers under semi-controlled conditions in container experiments, (ii) field trials conducted at three locations, in order to investigate nitrogen use efficiency and yield performance in different fertiliser levels and crop rotation scenarios, as well as (iii) pre-anthesis trials to investigate N-uptake and N-utilisation within plant compartments under different water regimes in early growth stages.

Poster 3

***hap3A.1* is a novel genetic determinant for grain yield and stability performance in wheat**

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Wheat is one of the major crops worldwide, providing 20% of the daily protein and food calories for 4.5 billion people. Nevertheless, considering that wheat production levels have not met the demand in the recent years, the annual wheat yield increases must rise from the current level of below 1% to at least 1.6%. Therefore, a better understanding of the genes controlling variations in wheat grain yield and stability can accelerate the required crop improvements. In this study, we characterized a diversity panel of 213 wheat varieties that have been released in the past 50 years to identify key genetic factors contributing to improve grain yield (GY) and yield stability performance (GYSP). The diversity panel was evaluated at six different locations in Germany across three growing seasons (2015-2017) under LN\_NF (Low-Nitrogen, no-fungicides), HN\_NF (High-Nitrogen, no-fungicides), and HN\_WF (High-Nitrogen, with fungicides) crop management systems (CMS). Results from data analyses showed that newly released (2004-2013) wheat cultivars had higher GY potentials and are more stable than the older cultivars (1946-1977) across 18 environments in each of the three CMS evaluated. This offers promising prospects towards simultaneous improvement of wheat grain yield and its stability performance. The Genome-wide association study using SNP marker data from genotyping analysis (Axiom 135K SNP arrays) identified 27 marker-trait associations ( $R^2 = 8.56$  to  $16.82\%$ ) to be associated with GY and GYSP across CMS. Among them are two SNP-clusters on Chr. 3A and Chr. 7B. The former has an effect on GY and GYSP under LN\_NF and HN\_NF, while the latter only affected GYSP under HN\_WF. Haplotype analysis identified one haplotype block (*hap3A.1*) spanning 13.22 kb (in RefSeq v1.0) on Chr. 3A region containing four genes: *CCB3*, *ABC*, *MPPA*, and *LRR1*. Similarly, the SNPs identified on Chr. 7B co-localized with phytotoxin resistance genes. Taken together, the high-resolution physical map, the association of Axiom-capture SNPs with GY and GYSP under different CMS, and the known roles the orthologous genes play in other cereals suggest that the *LRR1* is the most likely candidate gene in the *hap3A.1* region for GY and GYSP in wheat.

Poster 5

**Genetic basis of the trade-off between grain yield and baking quality in winter wheat**Manuel Geyer<sup>1</sup>, Lorenz Hartl<sup>1</sup>, Volker Mohler<sup>1</sup><sup>1</sup>Bayerische Landesanstalt für Landwirtschaft Manuel Geyer     manuel.geyer@lfl.bayern.de



Grain yield and baking quality are two major targets in wheat breeding. Baking quality is mainly determined by the content and composition of proteins and kernel hardness. The often-observed inverse relationship between grain yield and grain protein content is therefore a limiting factor for the development of high-yielding wheat varieties with good baking quality. Previous efforts have suggested several strategies to simultaneously select for grain yield and baking quality. However, the underlying genetic factors controlling the negative correlation between these traits have been largely unknown. In order to gain a better understanding of this relationship, the present study was aimed at the identification and characterization of quantitative trait loci (QTL) affecting agronomic traits and baking quality using a multi-parental advanced generation intercross population representing the German winter wheat breeding pool. Simple interval mapping identified multiple QTL for grain yield parameters, direct and indirect quality traits, and the derived traits of grain protein yield and grain protein deviation. Among the detected QTL, several genetic regions were found to simultaneously affect grain yield parameters and baking quality to a significant extent. The identified loci were characterized regarding their effects on relevant traits, their possible physiological roles and their potential for wheat breeding. The findings of this study are the basis for targeted analysis of crucial QTL and a valuable resource to facilitate marker-assisted and genomic selection approaches for grain yield and quality parameters in wheat breeding.

Poster 7

## Determination of *HSP16.9* gene using allele-specific primer and membrane thermal stability in different wheat genotypes

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Plants have developed various mechanisms for heat-stress adaptation, including changes in protein metabolism, such as the induction of heat shock proteins (HSPs). HSPs, also known as molecular chaperones, regulate protein folding and assist newly synthesized proteins to achieve their native state. Also, they prevent non-specific aggregation of proteins and take part in protein refolding under thermal stress conditions. The main aim of this study is to analyze the germplasm of different wheat genotypes for the *HSP16.9* gene, which belongs to low molecular weight heat shock proteins, using a SNP-based allele-specific primer. As a research object, 51 wheat genotypes were used: 36 genotypes of bread (*Triticum aestivum* L.) and 15 genotypes of durum (*Triticum durum* Desf.), which are collected in the gene pool of the Research Institute of Agriculture (Baku). Total DNA was extracted by CTAB method. Allele-specific primers (HSP16.9F CAGCAATCAACACCACGATG / HSP16.9R TGCCACTTGTCGTTCTTGTC) were used for DNA amplification. Agarose gel electrophoresis of PCR products showed that a 197 bp fragment, which is considered to be diagnostic for the *HSP16.9* gene, was successfully synthesized in 73% of the studied genotypes (28 bread wheat genotypes and 9 durum genotypes). The expected fragment was not amplified in 6 samples of durum and 8 of bread wheat genotypes. At the same time, membrane thermal stability (MTS) was measured during heat stress. 7-day-old seedlings were subjected to heat stress for 5 minutes. In order to induce total electrolyte leakage from leaf tissues, plants were kept in a boiling water bath for 30 minutes. Then the total electrolyte leakage was recorded by a conductivity meter (Horiba Scientific). Relative to control indicators, during heat stress, a significant increase in membrane damage rate (MDR) was observed. MDR is a quantitative indicator that is directly proportional to thermal damage and an inverse indicator of thermal stability. Highest MDR was found in cultivar Shiraslan23 (39.53) and the lowest in Giymatli2/17, (0.31). The value of MDR is a relative measurement of electrolyte leakage induced by high temperatures, hence it can directly indicate membrane damage rate. In wheat plants, a direct correlation between resistance to high temperature and MTS was observed.

Keywords: wheat, heat stress, HSP, membrane damage rate, electrolyte leakage

Poster 9

## Expression divergence of the DREB transcription factor among contrasting wheat genotypes under drought stress

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*DREB*, the Dehydration Responsive Element (DRE)-binding proteins, belong to the superfamily of *AP2/ERF* plant transcription factors known to regulate diverse processes of plant development and stress responses. The expression level of the *DREB* transcription factor gene was examined under drought in wheat genotypes of Azerbaijani origin differing in drought resistance: two tetraploid wheats (*Triticum durum* Desf.), Barakatli 95 (tolerant), Garagylchyg 2 (sensitive) and two hexaploid wheats (*Triticum aestivum* L.), Azamatli 95 (less sensitive), Giymatli 2/17 (sensitive), as well as hexaploid Batis and the synthetic hexaploid wheat accession Syn022L. We exposed 12-day-old seedlings of all selected genotypes to drought inside a growth chamber. Expression of the *DREB* gene was analyzed at 7 days after stress (DAS), when the visible stress-related traits were observed. The transcript level of *DREB* was determined by qRT-PCR using the elongation factor 1 alpha (*EF-1 $\alpha$* ) gene as an internal control. The fold change in expression was determined according to the  $2^{-\Delta\Delta C_t}$  method. There was no difference in the *DREB* expression levels in various genotypes of the normally watered plants. The transcript levels of *DREB* were increased in all drought-exposed genotypes and significantly varied among species. The average expression levels of the studied gene showed that German wheat genotypes had the highest upregulation under drought stress conditions. *DREB* transcript levels in the Batis genotype increased 2.6 fold and in the synthetic wheat Syn022L, 1.9 times. A lower *DREB* level was found in Garagylchyg 2. Under stress conditions, the expression level of the studied gene increased almost to the same extent in Barakatli 95 and Azamatli 95. In general, under drought stress, the expression level of the *DREB* transcription factor gene in tolerant genotypes increased more than in drought-sensitive ones. At the same time, we measured the vegetation index NDVI (Normalized Difference Vegetation Index). The effect of drought on NDVI score at various genotypes was significant. NDVI decreased at 3 DAS, reaching the control, then increased to its maximum point at 5 DAS, and decreased significantly in all genotypes at 7 DAS. The maximum NDVI score was found for Barakatli 95 and the minimum for Syn022L, with scores of 0.43 and 0.28, respectively. The highest decrease occurred in the genotypes of German origin (1.3-fold). One of the interesting aspects is that at 7 DAS, when drought stress was more severe, a lower NDVI score was observed in the German genotypes having higher expression level of *DREB* gene.



Poster 11

**A stable and novel QTL on chromosome 3A induces early heading in winter bread wheat**Salma Benaouda<sup>1</sup>, Said Dadshani<sup>1</sup>, Jens Léon<sup>1</sup>, Agim Ballvora<sup>1</sup><sup>1</sup>University of Bonn Salma Benaouda  Benaouda@uni-bonn.de

The genetic potential of bread wheat to adapt to a wide range of different climatic conditions mostly depends on the allelic variation of genes regulating the crosswalk to move from the vegetative growth to reproductive stage. Regulation of heading date (HD) is of crucial importance for breeding modern wheat cultivars adapted to various environments, especially in the frame of global climate change. In this study, a worldwide association panel of 220 winter cultivars was grown for 3 years in seven different locations all over Germany. Key environmental factors involving temperature, global radiation, growing degree days, precipitation and day length were measured in winter and spring for a more accurate correlation analysis with HD. We observed that HD is delayed by up to 20 days in locations with higher latitudes compared to lower latitudes. Furthermore, we found that early HD is negatively affected by increasing spring temperatures in environments with shorter day lengths whereas the effect is strongly positive in environments with longer days. By contrast, the effect of precipitation to heading is the other way around. To decipher the genetic adaptation to these environmental conditions, Genome-Wide Association Studies were conducted using 135 K SNP chip. A stable and novel QTL on chromosome 3A was detected in all locations explaining 30-58% of genetic variance. Additionally, a large number of detected environment specific loci indicate that they might play a role in fine-tuning to local climatic conditions. Taken together, our results mark a further step towards elucidation of the genetic basis for adaptation of HD to environmental conditions in wheat.

Poster 13

## **Sustainable increase of nitrogen and phosphorus efficiency in winter wheat through effective root-soil interactions**

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Nitrogen and phosphorus are essential macronutrients, and their sufficient availability is essential for qualitative and quantitative crop production. However, due to limited availability of resources, environmental regulations, and economic issues, nitrogen and phosphorus use efficiency are of great importance to secure or to increase the yield potential of modern wheat varieties. Particularly, plant root morphology is of great importance for accessibility and uptake of nitrogen and phosphorus. We conducted rhizotron experiments at Jülich Plant Phenotyping Center to decipher adaptation mechanisms of wheat plants to nitrogen and phosphorus deficient soils using the phenotyping platform GROWSCREEN-Rhizo, measuring daily development of multiple root traits like length of seminal and lateral roots, root convex hull area, etc. We observed diversity among the members of the tested association panel with a range of adaptation mechanisms to nutrient deficient soils.

To further conceive our knowledge of these mechanisms, the development of root traits was observed along the rhizotrons up to 90 cm below ground surface in the time course of 15 days. Here, we were able to detect a hotspot for lateral roots 5 - 20 cm below the ground surface to play a significant role with respect to genotype by treatment interaction under nitrogen deficient conditions. Differently, under P deficient conditions, the development of seminal roots 0 - 10 cm below ground surface was of major importance during the seedling stage, whereas lower, deeper root levels gain more importance by the development of plants.

Genome-wide association studies using SNP genotypic data from 135K Affymetrix array analysis will be conducted to gain insight into the molecular background of regulation of root response to nitrogen and phosphorus deficient conditions in wheat. The detection of genomic regions involved in the regulation of root traits in terms of nutrient uptake will accelerate the breeding of future wheat varieties with increased nitrogen and phosphorus use efficiency.

Poster 15

## Association analysis in lines derived from winter wheat CCPs—comparing four different populations stratification methods

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Introduction: Composite cross populations (CCP) have an excellent capacity for self-regulation allowing for overall reduction of external inputs while maintaining or increasing overall system output including ecological services. This study attempts to use wheat lines selected from CCPs in order to test the applicability as a diverse, but still restricted population for GWAS.

Materials and Methods: DNA was isolated from 184 CCP lines derived from two winter wheat CCPs and genotyped using a 20k wheat SNP array (TraitGenetics, Infinium Ultra HD). The genotyping data, together with phenotypic data were used to associate marker alleles to trait expressions by GWAS. Several statistical methods for association were used: general linear model (GLM), mixed linear model (MLM), multi-locus mixed model (MLMM), fixed and random models circulating probability unification (FarmCPU) using GAPIT version 3 (Wang and Zhang, 2019) including kinship and covariate matrix. Covariates were calculated using principal component analysis (PCA), principal coordination analysis (PCoA), interval as well as M-spline multiple dimensional scaling (iMDS/msMDS) with Torgerson initial configuration (TC; de Leeuw and Mair 2009), and uniform manifold approximation and projection spectral embedding (UMAP-Sp; McInnes et al. 2018) based on 4,676 filtered SNPs or on a simple match distance matrix of that SNP information. Altogether, 51 combinations were compared by calculating Pearson correlation coefficients of the p-values yielded from the GWAS models, converted to Euclidean distances. As an example, results for plant height are described.

Results: In general, all methods tested might explaining population structure (PS) in wheat CCPs. However, for plant height, UMAP yielded the best results for correcting PS used in GLM. PCA outperformed MDS-based PS methods. As for MLM- and MLMM-based models, little differences were observed between PS configurations. In contrast, FarmCPU-based models tend to be conservative: the correction for PS with PCA tends to be too strong. The results of GLM-, MLM, and MLMM-based models tend to cluster together, whereas FarmCPU shows different outcomes.

Conclusions: The preliminary results are promising and show a potential to use covariate methods for GWAS when analysing data derived from diverse wheat CCP lines. Therefore, further investigations and comparison with different environments and methods are needed.

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Poster 17

## High-resolution mapping of rachis nodes per rachis, a critical determinant of grain yield components in wheat

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Increasing grain yield in wheat is a key breeding objective worldwide. Several component traits contribute to grain yield with spike attributes being among the most important. In this study, we performed a genome-wide association analysis for 12 grain yield and component traits measured in field trials with contrasting agrochemical input levels in a panel of 220 hexaploid winter wheats. A highly significant, environmentally consistent QTL was detected for number of rachis nodes per rachis (NRN) on chromosome 7AL. The five most significant SNPs formed a strong linkage disequilibrium (LD) block and tagged a 2.23 Mb region. Using pairwise LD for exome SNPs located across this interval in a large worldwide hexaploid wheat collection, we reduced the genomic region for NRN to a 258 Kb interval containing four of the original SNP and six high-confidence genes. The ortholog of one (TraesCS7A01G481600) of these genes in rice was *ABBERANT PANICLE ORGANIZATION1 (APO1)*, which is known to have significant effects on panicle attributes. The *APO1* ortholog was the best candidate for NRN and was associated with a 115 bp promoter deletion and two amino acid (C47F and D384N) changes. Using a large worldwide collection of tetraploid and hexaploid wheat, we found 12 haplotypes for the NRN QTL and evidence for positive enrichment of two haplotypes in modern germplasm. Comparison of five QTL haplotypes in Australian yield trials revealed their relative, context-dependent contribution to grain yield. Our study provides diagnostic SNPs and value propositions to support deployment of the NRN trait in wheat breeding.

Poster 19

## Changes in some antioxidant enzymes activity of wheat genotypes under drought stress during leaf senescence

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The study of physiological aging of the flag leaf, which has a key role in the absorption of solar energy during photosynthesis in wheat, is one of the most important parameters for ensuring high productivity under stress. The aim of the study was to determine the activity in dynamics of the ascorbate peroxidase (APO) and catalase (CAT) enzymes in wheat, which is one of the main components of antioxidant defense systems during flag leaf senescence. For this purpose, Vugar (drought tolerant) and Tartar (susceptible) genotypes were used as a research object. The plants were grown under natural conditions, and then exposed to drought stress by the stopping of watering. Measurements were carried out at 6 points at intervals of 7 days after the appearance of a flag leaf. The obtained results showed that APO activity was highest in the youngest flag leaf in normal-watered plants, and remained almost constant throughout vegetation. A significant increase in APO activity was observed at the end of growth, when signs of senescence appeared. In tolerant genotype Vugar, during leaf senescence, APO activity was increased and reached maximum level at the 35<sup>th</sup> day, then at the 42<sup>nd</sup> day, due to senescence degradation processes, the rate of activity falls down dramatically. However, susceptible genotype Tartar shows 2 maxima of APO activity during senescence: the first peak was observed on the 21<sup>st</sup> day, and the second one on the 35<sup>th</sup> day. The activity of catalase differed from APO activity dynamics during leaf senescence. The activity of this enzyme has minimal value during the initial periods of vegetation in irrigated plants. Catalase activity reaches the maximum level at the 21<sup>st</sup> day in the tolerant genotype. In the sensitive genotype, the activity of catalase shows 2 peaks: 1st peak with maximum activity on the 21<sup>st</sup> day, the second on the 35<sup>th</sup> day. Minimal activity of CAT was observed at the end of senescence.

Poster 21

## **MAGIC-RESIST - Identification and mapping of resistances against fungal diseases in the MAGIC-WHEAT population WM-800**

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Detecting new genes for disease resistances in agricultural crops is crucial for sustainable crop production. Phenotyping large mapping populations for detection of quantitative resistances is time- and resource-consuming, so that new approaches appear highly necessary. In our study, we combine classical and new phenotyping approaches with modern statistical methods.

The targets are to

- evaluate the potential of unmanned aerial vehicle (UAV) based phenotyping to supplement ground phenotyping,
- detect quantitative trait loci (QTL) for leaf rust, stem rust and *Fusarium* resistances via genome-wide association analysis (GWAS),
- estimate the QTL effects to support selection decisions in practical breeding.

We are phenotyping the MAGIC (multi-parent advanced generation intercross) winter wheat population WM-800 (Sannemann et al. 2018) in field trials in Halle and Quedlinburg in 2018/19 and 2019/20. In all environments, we use a randomized complete block design with two to three replications and inoculation with fungal pathogens (leaf rust, stem rust, *Fusarium*). Examined traits are disease severity, heading date, plant height, ear weight and thousand grain weight. In Halle in 2018/19, repeatabilities were high for powdery mildew (PM) infestation (0.88), plant height (0.91) and heading (0.93) and on a medium level for stripe rust (SR; 0.64), leaf rust (LR; 0.60) and *Fusarium* ear infestation (FUS; 0.49). Leaf coverage by PM was considerable (mean=3.2%; SD=2.1%). Despite inoculation, infestation levels were low for SR (0.15%; SD=0.53%), LR (1.4%; 2.0%) and FUS (1.2%; 0.91%). The applied UAVs carry a red-green-blue camera and a multispectral camera with a thermal channel, which enables modeling relationships between ground-scored disease severities and UAV-derived spectral data. Genotypic data were collected through Illumina wheat 15k SNP array and Affymetrix 135k array analysis, carried out by TraitGenetics, Gatersleben. The assays delivered 27,685 informative SNPs in total with physical positions according to the Refseq 1.0 (IWGSC). A GWAS with two-year and two-location data will be performed with multiple linear regression analysis.

Reference

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Poster 23

**Fine-mapping *Qfhs.ifa-5A* revealed two tightly linked QTL both affecting resistance against initial infection and anther extrusion**Maria Buerstmayr<sup>1</sup>, Barbara Steiner<sup>1</sup>, Christian Wagner<sup>1</sup>, Magdalena Ehn<sup>1</sup>, Andrea Danler<sup>1</sup>, Babor Eshonkulov<sup>2</sup>, Hermann Buerstmayr<sup>1</sup><sup>1</sup>Department of Agrobiotechnology, Institute of Biotechnology in Plant Production, University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Str. 20, 3430 Tulln, Austria;<sup>2</sup>Agricultural University of Uzbekistan, Samarkand, Uzbekistan Maria Buerstmayr  maria.buerstmayr@boku.ac.at

Resistance to *Fusarium* head blight (FHB) is of great importance in modern wheat varieties in many wheat growing areas worldwide. Numerous FHB resistance QTL have been identified so far, however, only few have been validated and fine-mapped. *Qfhs.ifa-5A* (Buerstmayr et al. 2003) is among the best-validated FHB resistance QTL, and it has been introgressed in adapted breeding material showing consistent, strong reduction of disease severity. It predominantly contributes resistance to fungal entry with the favorable allele descending from the highly *Fusarium* resistant wheat cultivar Sumai-3. Fine-mapping *Qfhs.ifa-5A* revealed the underlying genetic control of the FHB resistance as highly complex and separated the *Qfhs.ifa-5A* interval into two QTL. The major effect QTL *Qfhs.ifa-5Ac* mapped across the centromere and the smaller effect QTL *Qfhs.ifa-5AS* mapped to the distal half of 5AS. Although *Qfhs.ifa-5Ac* and *Qfhs.ifa-5AS* were delimited to genetic intervals as small as 0.1 and 0.2 cM, the corresponding physical distances were large and comprised 44.1 Mbp and 49.2 Mbp, respectively. Sumai-3 alleles at either QTL significantly improved resistance and reduced anther retention, suggesting a pleiotropic effect of anthers on *Fusarium* resistance. This hypothesis was further supported by an experiment using Remus and its near isogenic line NIL3 carrying the *Qfhs.ifa-5A* alleles. Through removing anthers, resistant NIL3 and susceptible Remus became almost equally resistant and were significantly less diseased than their control variants without anther manipulation at early time points after inoculation. At late time points, the positive effect of anther removal became smaller for Remus and disappeared completely for NIL3. Results clearly showed that absence of anthers primarily enhanced resistance to initial infection, while it did not protect plants from fungal spreading within the spikes.

The knowledge of increasing anther extrusion as a major resistance component of *Qfhs.ifa-5A* will assist in gene identification by suggesting candidates for further analysis/reverse genetics.

**Acknowledgement**

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**Reference**

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Poster 25

## Isolating the wheat gene enhancing mycotoxin detoxification at the major *Fusarium* resistance QTL *Fhb1* – a progress report

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*Fhb1* is the best validated and most prominent *Fusarium* resistance QTL of wheat, it confers not only strong resistance to spreading of the disease but also to the major mycotoxin deoxynivalenol (DON) by conjugation into the non-toxic DON-3-O-glucoside (D3G)<sup>[1]</sup>. While *Fhb1* is already deployed in elite germplasm, the gene encoding resistance against DON has not yet been characterized. We have established the genomic sequence of the *Fhb1* region from the donor line CM-82036 and fine-mapped the QTL to an 860 kb interval comprising 28 candidate genes<sup>[2]</sup>. Mutant populations of CM-82036 were used for gene validation: TILLING identified mutant lines for eleven candidates - no loss of resistance phenotypes led to the rejection of all tested as the causal *Fhb1* gene. Forward genetics screening of 1,200 gamma-radiated and 2,500 EMS mutant lines revealed four and three DON- and *Fusarium*-susceptible mutants, respectively. Genotypic characterisation of the gamma-radiated lines detected deletions for all four susceptible lines covering the complete *Fhb1* interval confirming presence of active resistance gene(s) at the QTL. The susceptible EMS-mutants were sequenced for the candidate genes, resulting in 2-3 SNPs per line, but no common factor was found. However, for all three susceptible lines, F<sub>2</sub> co-segregation analysis confirmed the QTL region as responsible for the altered phenotype after *Fusarium* and DON treatment. DON and D3G contents were determined to compare conversion rates of CM-82036 and the susceptible mutants, detecting about 15 times higher amounts of D3G compared to DON in the wild-type lines whereas in the susceptible mutants including F<sub>2</sub> progenies more than 40% of the DON was still present. DON and *Fusarium* infiltrations gave similar DON/D3G ratios proposing that DON detoxification is regulated by mutations in the *Fhb1* interval controlling both traits. Thus, to detect all mutations in the QTL region, the susceptible mutants and CM-82036 were flow-sorted for 3B chromosomes and sequenced. A chromosome assembly of the wild-type was established (130,108 scaffolds, 722.6 Mb) and sequencing reads of the mutants were mapped to it and to the 1 Mb QTL interval. Following the MutChromSeq approach<sup>[3]</sup> we identified hundreds of scaffolds with SNPs in all three mutants; two were positioned in the *Fhb1* interval representing targets for further analysis.

Acknowledgment: funded by Austrian Science Fund: SFBF3711

References:

[1] Lemmens et al. 2005, DOI: 10.1094/MPMI -18-1318; [2] Schweiger et al. 2016, DOI 10.1007/s00122-016-2727-x; [3] Sánchez-Martín et al. 2016, DOI 10.1186/s13059-016-1082-1





Poster 27

## **Fusarium head blight resistance in winter wheat: Insights from genome-wide transcriptome analysis**

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*Fusarium* head blight (FHB) is a major disease in wheat and many other small-grain cereals, which can cause dramatic yield losses along with a strong reduction in grain quality due to mycotoxin contamination. Quantitative genetic variation for FHB resistance is evident, but much remains unknown about the underlying transcriptomic defense mechanisms to fungal invasion in wheat. We used RNA-sequencing technology on a diverse winter wheat panel of 96 genotypes for a detailed investigation of the transcriptomic response to *Fusarium graminearum* infection. This diversity panel included breeding lines, European elite cultivars, as well as experimental lines derived from the exotic resistance donor Sumai3, and therefore shows huge variation in FHB resistance. Differentially expressed gene (DEG) analyses showed that gene expression was strongly influenced by fungal infection, while numerous *Fusarium*-responsive genes could be identified for the RNA-sampling time point 48 hours after inoculation with most of them being upregulated. A total of 422 DEGs were detected in all 96 genotypes, suggesting a general transcriptomic response to fungal attack. Interestingly, numerous kinases were up-regulated under *Fusarium* treatment, which have been reported to play an important role in signaling pathways. Furthermore, *DUF538* family genes that have been suggested to be involved in chlorophyll degeneration were strongly upregulated. Enriched GO terms of the DEGs were clearly associated with stress response, fungal defense, signaling, and cell communication. We further discovered that the *Fusarium* response in different resistant groups is similar, with slightly more DEGs in the moderately resistant and very susceptible groups. Additionally, an eQTL-mapping analysis revealed numerous *Fusarium* associated eQTL with substantially more distant than local eQTL. These regulatory loci and *Fusarium* responsive genes are potentially useful for providing additional insight into *Fusarium* head blight resistance in wheat and for unraveling its genetic mechanisms on the expression level.

Poster 29

## Improving *Fusarium* head blight resistance in durum wheat through introgression of resistance alleles from relatives

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The demand for pasta has increased, therefore durum wheat production is also demanded to rise. However, durum wheat is very susceptible to *Fusarium* Head Blight (FHB) and infection with *Fusarium* results in yield losses and quality deterioration, as well as mycotoxin contamination that is harmful to humans and animals consuming it. Relying on fungicides continuously has an impact on environmental pollution and exterminates beneficial microorganisms. Resistance breeding to support the development of FHB resistant durum cultivars is an urgent need, which is still hampered by the limited genetic variation in durum wheat. The objective of this research is to broaden the narrow genetic basis for FHB resistance in durum wheat by introgression of resistance alleles from wild and cultivated relatives. Therefore, we developed 900 multi-parental breeding lines with resistance alleles derived from *T. aestivum*, *T. dicoccoides*, and *T. dicoccum*. These lines were phenotyped in the field over three seasons for FHB resistance using spray inoculation. Lines show broad variation for FHB resistance, including moderately resistant lines, although a strong correlation between plant height and severity was found. Nevertheless, short and moderately resistant lines could be identified. In addition, the lines will be genotyped to enable genome-wide QTL mapping and to elucidate the genetic control of FHB resistance in this population. The moderately resistant durum lines represent highly breeding-relevant material to achieve higher levels of resistance in elite durum germplasm.

Poster 31

***Fusarium graminearum* and deoxynivalenol resistance in *Aegilops tauschii***Rizky Psthika Kirana<sup>1,2</sup>, Barbara Steiner<sup>1</sup>, Michel Sebastian<sup>1</sup>, Kumar Gaurav<sup>3</sup>, Sanu Arora<sup>3</sup>, Marc Lemmens<sup>1</sup>, Brande B.H Wulff<sup>3</sup>, Hermann Buerstmayr<sup>1</sup>

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The bread wheat progenitor, *Aegilops tauschii*, ( $2x=2n=14$ , DD), is a promising source of resistance genes for use in wheat improvement (Arora et al. 2019). *Fusarium* Head Blight (FHB), caused by *Fusarium graminearum*, is an important disease in wheat because it not only damages plants but also produces Deoxynivalenol (DON), which is the major *Fusarium* mycotoxin with high toxicity effect. This study aims to evaluate the variation of *Fusarium* and DON resistance within a diverse panel of *Ae. tauschii* accessions. Therefore 152 *Ae. tauschii* accessions were point-inoculated by pipetting *F. graminearum* spores in a single spikelet per head and the spreading of the disease was determined. In parallel, the heads were also infiltrated with DON and the mycotoxin-induced bleaching symptoms were recorded. We revealed broad variation of the disease spreading within the spike, although no highly resistant accessions were identified and the fungal spreading promoted early 'spike shattering', thereby complicating disease phenotyping. For DON resistance, less variation was found, most of the accessions showed bleaching symptoms only on the treated spikelet, but for nine genotypes the DON infiltration resulted in severe bleaching symptoms. DON contents and the conjugated non-toxic DON-3-O-glucoside contents of treated heads will be determined to analyze DON detoxification in *Ae. tauschii*. This winter the greenhouse experiment will be repeated. In addition, available extensive sequence data of the accessions may allow mapping of FHB and DON resistance genes and moreover the identification of candidate genes.

## Reference



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Poster 33

## Common bunt resistance in winter wheat – a cross-chromosome journey

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Common bunt, a seed-borne disease caused by the fungi *Tilletia tritici* and *T. laevis*, seriously affects grain yield and quality in wheat. Only one (*Bt10*) of 16 race-specific resistance genes is currently widely used in breeding programs. Since the majority of bunt resistant material is not suitable for Austrian agriculture, there is an urgent need to incorporate *Bt*-genes into regionally adapted cultivars. Marker-assisted and genomic-assisted selection can be applied to introgress resistance alleles from wheat relatives or landraces into elite breeding lines and eliminate unfavourable properties thereby introduced through linkage drag.

To pave the way for incorporating exotic resistance sources into breeding lines adapted to mid-European growing conditions, two different experiments are conducted at IFA Tulln:

a) Two RIL populations derived from crossing winter wheat genotypes PI166910\*Rainer and 702-1102C\*Rainer were phenotypically screened for common bunt resistance using artificial seed inoculation at IFA Tulln in 2019. PI166910 is the carrier of bunt resistance gene *Bt11* and probably *Bt9* and 702-1102C carries either *Bt8* or *Bt9* (Anders Borgen, personal communication) while Rainer is highly susceptible to common bunt. Steffan et al. (2017) mapped *Bt9* to the long arm of chromosome 6D. We selectively genotyped RILs with KASP markers positioned between 400 and 470 Mbp on chromosome 6D and SSR markers indicative for the QTL interval of *Bt11* on chromosome 3B. Preliminary results show that 702-1102C and PI166910 carry the *Bt9* gene and that PI166910 contains an additional yet not known resistance gene (possibly *Bt11*).

b) A backcross-population consisting of BC<sub>2</sub>F<sub>2</sub>- and BC<sub>3</sub>F<sub>1</sub>-lines was developed from initial crosses of elite cultivars to non-adapted common bunt resistance donors by repeated backcrossing. The donor-lines (Blizzard, Bonneville and the Turkish landrace PI119333) confer resistance via 5 different QTL located on chromosomes 1A, 1B, 4B, 7A and 7D. PI119333 contributes *Bt12* on 7D. By MAS with KASP-markers, plants heterozygous at one or more resistance loci were selected, selfed and genotyped with GBS-markers. Depending on genomic predictions and the amount of BC<sub>2</sub>F<sub>2</sub>-seeds, a selection of lines will be propagated and subjected to artificial infection in 2021.

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Poster 35

## Insights into the genetic control of flowering time based on a worldwide series of field trials with the barley NAM population HEB-25

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The decision to initiate flowering is maybe the most critical switch during plant development. The plant has to perceive and respond to key environmental factors to ensure that flowering does not occur under adverse and detrimental conditions. Thus, during evolution, several processes of genetic adaptation evolved, enabling plants to flower at an adequate time point. In the present study, we investigated flowering time of the large wild barley nested association mapping (NAM) population HEB-25 in worldwide field trials to determine both environment-dependent and environment-independent genes controlling flowering time. For this purpose, seven environmentally different locations were chosen: Dundee (UK), Halle (Germany), Merchouch (Morocco), Gilat (Israel), Dubai (United Arab Emirates), Amlaha (India) and Charlick (Australia). They mainly differed for day length and temperature regime during the vegetation period. Flowering time varied strongly between locations, ranging from an average flowering time of 69 days after sowing in Germany to 114 days in Australia. Correcting flowering time for temperature resulted in a harmonization of flowering time, separating the locations based on the photoperiod into long-day (UK and Germany) and short-day conditions. The genome-wide association study (GWAS) revealed five major QTL with worldwide relevance. While *HvCEN*, *GA2Ox2* (*sdw1/denso*) and *HvFT1* (*Vrn-H3*) were detected with similar effects in all locations, *Ppd-H1* was exclusively found in long-day locations (UK, Germany), while *Vrn-H1* had the largest impact in heat-prone locations (United Arab Emirates, India). Different wild barley alleles could be distinguished based on their impact on flowering time between different environments. The knowledge gained will assist to further unravel the control of flowering time and its dependency on environmental factors. Moreover, it will assist to fine-tune flowering time of barley in regions severely affected by climate change.

Poster 37

**Broadening the genetic basis for hybrid breeding in winter barley**Timm Bernhard<sup>1</sup>, Rod Snowdon<sup>1</sup>, Benjamin Wittkop<sup>1</sup><sup>1</sup>Department of Plant Breeding, Justus Liebig University, Giessen Timm Bernhard  [tim.bernhard@agr.uni-giessen.de](mailto:tim.bernhard@agr.uni-giessen.de)

Winter barley (*Hordeum vulgare* L.) is the third most important crop in Germany, mainly used for animal fodder. As an autogamous cereal, the majority of registered barley varieties are true breeding inbred lines. However, breeding efforts today are tending towards the incorporation of hybrid varieties, which combine higher yields with a better yield stability and stress tolerance.

However, the market share of barley hybrid varieties is still relatively low, mainly due to small yield advantages resulting in a lower commercial heterosis. The yield advantage of hybrids is caused by the heterosis effect, which is generally correlated with the genetic distance of the parental lines. Therefore, a prerequisite for hybrid barley breeding is the division of parental lines into heterotic pools based on their genetic distance and combining ability. However, due to the autogamous nature of barley, line breeding has resulted in elite lines being closely related among breeders and countries, showing insufficient genetic distance.

In this regard, we crossed winter barley restorer lines (WBR) with spring barley elite lines (SB). The resulting WSBR-DH-populations were genotyped with the 50k Illumina SNP-Chip and selected for 6-rowed spikes, winter hardiness, vernalization requirement and existence of the restorer gene. Subsequently, these selected novel barley genotypes were crossed with winter barley cytoplasmic male sterile (CMS) mother lines. The derived test-hybrids, together with their parental lines, were evaluated in multi-locational yield performance trials to generate data for the calculation of the combining ability, heterosis and hybrid performance. Evaluation of the genetic distances of the tested WSBR-DH-populations (n=500) showed a clear distinction between clusters containing the WBR x WBR and the WBR x SB lines, respectively, demonstrating a successful broadening of the genetic diversity within the winter barley breeding material. Thus, introgression of spring barley germplasm can be advantageous in terms of broadening the genetic basis, but, as observed in the performance trials, an adaptation of the parental lines to the target environment is crucial for an enhanced hybrid performance.

Poster 39

## Quantification of root lesion nematodes by RT-qPCR in the roots of cereal plants

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Root lesion nematodes (RLN) *Pratylenchus neglectus* and *P. penetrans* are causing severe damage in German barley production. Because of climate change, damages caused by RLN are expected to increase and no treatment is both efficient and environmentally advisable. Since the assessment of RLN in the field as well as in the greenhouse is cumbersome and time-consuming, this pest has been largely disregarded by European cereal breeders so far. Therefore, a rapid method to quantify the root lesion nematodes in the infected roots is needed. In this study, we are aiming to develop a sensitive and reliable diagnostic method for species-specific quantification of *P. crenatus*, *P. penetrans*, *P. neglectus*, and *P. thornei* in infected roots. We established a protocol for the isolation of DNA from infected roots. Using species-specific primers, we could successfully amplify and quantify nematode DNA in the roots of infected plants. In the initial experiment, we found a lower  $C_t$  value for the susceptible control line (Valentina) compared to the resistant line (Beysehir). This was verified by the number of nematodes counted via a conventional microscopic method where the number of nematodes was lower in the resistant line compared to the susceptible one. We are performing a greenhouse experiment with 50 DH lines from the Beysehir-Valentina population with contrasting resistances infected with *P. neglectus* to validate the PCR protocol. We will aim to measure RLN infections only by RT-qPCR in the future, which will enable routine measurements to select resistant plants among large segregating populations, and to measure the abundance of nematodes in farmer's soils.

Poster 41

## **A systemic approach to identify the gene underlying flowering-delaying epistatic QTL “*Hvheading*”**

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Identification of the genes controlling flowering time can assist in understanding the mechanisms that improve yield due to its high correlation with grain yield. The aim of this study was to identify the gene underlying a novel flowering-delaying epistatic QTL, “*Hvheading*”, with no requirement for recombination or near isogenic lines using an RNA sequencing (RNA-seq) approach. For this purpose, we selected a comparable pair of DH lines from a spring barley MAGIC population that have the same background regarding major flowering time genes, and one of them carries the haplotype from a causative parent in *Hvheading*. Microscopic phenotyping of apex development and differential expression analysis using RNA-seq and real-time PCR was performed for apex and leaf tissue. Apex phenotyping detected the flowering-delaying effect of *Hvheading* starting after apex vegetative-to-reproductive transition. Our results showed that the region identified by RNA-seq spans an interval of 4.2 Mbs and 6.74 Mbs, which contain 60 and 82 genes for apex and leaf tissue, respectively. The detected region contained 6, 11 and 8 differentially expressed high confidence genes for apex tissue and 7, 12 and 17 differentially expressed high confidence genes for leaf tissue for time-points one, two and three, respectively. In apex tissue, the strongest differentially expressed genes that also showed strong difference among time points were transcription elongation factor *Spt6* and nuclear transcription factor Y subunit C-3, which were also present among the genes in leaf tissue. The RNA-seq results were validated by performing expression analysis using RT-PCR on another set of samples. Further studies are needed to elaborate the role and function of these genes in the flowering pathway of barley.

Keywords: Barley, RNA-seq, flowering time, novel QTL, MAGIC population, gene identification





Poster 43

## QTL mapping and genome-wide association mapping of root lesion nematode resistance genes in barley

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Root lesion nematodes (RLN) are causing severe damage in cereal production worldwide. Narrow crop rotation, early sowing dates, and mild winters, which are expected due to climate change, increase the damage by this pest. So far, because of difficulties in phenotyping, very limited information is available about the genetic mechanism behind RLN resistance in barley. We focus on two pests, which are frequently found in German farming fields, *Pratylenchus neglectus* and *Pratylenchus penetrans*. Previous studies have identified major QTLs, but with low resolution. We sequenced 500 DH-lines from a population between the susceptible accession Valentina and the resistant accession Beysehir. As a result, we identified 44,411 SNP markers, which allow a more precise mapping of the resistance QTLs for RLN resistance. The phenotyping of *P. neglectus* resistance was done in a greenhouse with six plants per line inoculated with 1000 nematodes in independent experiments. QTL mapping using the GBS data and the phenotypic data is currently ongoing. In addition, we are performing a GWAS with 425 barley accessions, which had been tested with *P. neglectus*. All accessions were sequenced, so that we are expecting close marker-trait associations. Both approaches will allow us to tightly map RLN resistance QTL and to develop molecular markers for marker-assisted selection, which is the only way to select resistant genotypes during a breeding program. Moreover, we are aiming to clone genes from major QTL to get insight into the molecular mechanisms of plant-nematode interaction.

Poster 45

## Analysis of population structure and genetic diversity within hybrid rye elite breeding component lines

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Rye remains to this day an important crop in the northern temperate zone, serving as a cost-effective feed source for pig fattening and as a key constituent in the Northern European diet. In addition, as a result of the recent years of devastating yellow rust epidemics in triticale and extreme, dry summers, cultivation of rye is experiencing a renewed interest. At the onset of this market development Nordic Seed A/S acquired a German hybrid rye breeding branch to expand their existing cereal breeding portfolio and meet the growing demand. Genomic-based breeding techniques are a common practice at Nordic Seed, thus the acquisition led to a comprehensive undertaking to not only phenotype the acquired elite hybrid rye breeding material, but likewise to dissect their genetic background. In this initial study, 376 lines belonging to the three component populations, *i.e.* restorer, maintainer and cytoplasmic male sterile (CMS), involved in hybrid rye breeding, were genotyped on a pre-designed 15K<sub>WHEAT</sub> + 5K<sub>RYE</sub> SNP array. Filtration for quality parameters led to 4419 polymorphic markers, distributed homogeneously throughout the rye genome. Fundamental calculations of genetic characteristics were done, such as the level of homozygosity, inbreeding coefficient ( $F_{IS}$ ) and the fixation indices ( $F_{ST}$ ) as a measure of the genetic differentiation between populations. Distinct genetic separation between the populations is key to obtaining and enhancing heterosis in hybrids. This separation could readily be confirmed in a principal component analysis, with accessions portraying a clear segregation in accordance to their population. The impact of recent years of intensive breeding effort in the restorer population was likewise evident, showing a substantially higher genetic diversity than observed in the maintainer and CMS population. This pattern was likewise evident in the neighbor-joining dendrogram and admixture model of inferred ancestry.

Analysis of linkage disequilibrium (LD) showed clear discrepancy between the populations, with LD being substantially lower for the restorers. Consequence of this insufficient marker density became evident in a Genome wide association study for the discovery of brown rust resistance linked markers. Despite a restorer population consistently segregating for resistance in a field trial at three locations, no markers could be linked to the resistance. This discovery has led to a pursuit to enrich the SNP chip with additional restorer markers. Conclusively, this study demonstrates the importance of understanding the genetic background of elite breeding material prior to the implementation of genomic-based breeding techniques.

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## Exploring molecular markers in conjunction with traditional DUS traits for managing reference collections in European rye varieties

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Registration of new plant varieties requires successful testing for Distinctness, Uniformity and Stability, shortly called DUS-testing. Traditional DUS-testing is performed by assessing plant phenotype in large field trials, which is time-consuming and laborious. The rapid development of genotyping and sequencing technology allows to complement traditional DUS-testing with molecular markers. The International Union for the Protection of New Varieties of Plants (UPOV) has developed three models for using molecular markers in variety testing. We assessed the use of markers to manage reference collections of examination offices according to UPOV BMT model 2. A total of 3,728 plants from 82 different rye varieties, consisting of hybrid varieties, open-pollinated varieties (OPVs) and synthetic varieties, were genotyped with 5,234 SNP markers. To determine the genetic relationship of varieties, we applied several measures of genetic similarity like Roger's Distance (RD) and Discriminant Analysis of Principal Components (DAPC) to unravel the genetic structure of the examined varieties. Genetic uniformity of varieties was assessed by calculating the Coefficient of Variance (CoV). We then combined the genetic with the phenotypic data from DUS trials to test whether it is possible to define a relationship between genetic and phenotypic differences between varieties. Phenotypic data for 21 DUS traits from 71 rye varieties were analyzed with multivariate methods like Principal Component Analysis (PCA) and Multidimensional Scaling (MDS) and correlated with genetic distances. Our results show that varieties can be differentiated well using molecular markers and that differentiation can be achieved with reduced marker sets. However, unlike in other crops like barley, there is no correlation between phenotypic (based on DUS traits) and genetic distances suggesting that genome-wide marker-based distances are not a good substitute for phenotypic distinction of varieties based on DUS traits. With respect to uniformity, marker-based estimates show that CoV of hybrid varieties is larger than of OPVs. In conclusion, Roger's Distance is a suitable measure to distinguish varieties in marker-based DUS testing even with a small set of SNP markers, but the relationship to DUS-based distinctness is weak. CoV might be a suitable marker-based measure to define uniformity.

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## PEGASUS: Prediction and Exploitation of Gene bank Accessions – a study in Ugandan Sorghum

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*Sorghum bicolor* is a multi-use crop showing huge adaptive diversity across different climate zones of the world. Its high nutrient use efficiency, combined with strong resilience to high temperatures and drought, makes sorghum a climate-ready crop that can play a key role in agriculture worldwide. Uganda is one of only three countries in which all of the five basic races and ten intermediate races of sorghum are endemic (Gopal Reddy et al. 2002, *Intl. Sorghum Millet Newsl.* 43). Uganda's broad sorghum diversity reflects the diverse environments where the crop is grown, from extremely arid and semi-arid zones in eastern and northern Uganda to the very cool highlands in south-western Uganda. The germplasm from the highlands possibly carries potentially useful alleles for cold stress adaptation, a major trait of interest for Germany and other temperate areas of the world.

The Uganda National Gene Bank has conserved this sorghum diversity in a collection spanning more than 3000 accessions; however, this germplasm has not yet been fully characterized and evaluated, limiting its utilization for breeding. We have genotyped the collection using cost-effective, high-density DARTseq markers, in order to identify a core collection representing the entire diversity and establish genetic relationships to well-characterized global sorghum collections (e.g. Mace et al. 2013, *Nat Comm* 4: 2320). Furthermore, we are phenotyping the core set for traits associated with cold adaptation of sorghum to European conditions and yield, plant architecture and resistance-related traits under African conditions. The data will facilitate genomic predictions within the entire collection to identify potentially useful germplasm for crossing into international breeding programs. The results and experience from this project will help to design viable options for developing countries to more effectively manage their valuable plant genetic resources and identify useful diversity for local and global agricultural challenges.

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## **Long-term trends and genetic architecture of seed characteristics and grain yield components in triticale ( $\times$ Triticosecale Wittmack)**

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The aim of this study was to investigate long-term genetic trends and the genetic architecture of grain yield and its component traits in triticale. To this end, a panel of 846 diverse triticale genotypes was assessed for three agronomic and three seed shape- and size-related traits. We observed a high genotypic variation and a high heritability for all traits. Analyzing the development of these traits during the last decades revealed a continuous increase for grain yield and thousand-kernel weight, and a slight increase in seed width. The seed characteristics and thousand-kernel weight formed a complex of highly positively correlated traits. Genome-wide association mapping revealed many small-effect and a few moderate-effect QTL. The allele frequencies of the moderate-effect QTL followed the same temporal trends as observed for the phenotype. In line with the phenotypic correlations, we identified several pleiotropic QTL for grain yield, thousand-kernel weight, seed width and seed area. Our results illustrate the continuous progress achieved in triticale breeding and suggest that triticale seeds have been selected to be more spherical in modern cultivars.

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**Assembling the restructured genome of a novel synthetic *Brassica napus* with diverse genome donors**Mauricio Orantes Bonilla<sup>1</sup>, HueyTyng Lee<sup>1</sup>, Harmeet Singh Chawla<sup>1</sup>, Jun Zou<sup>2</sup>, Rod Snowdon<sup>1</sup><sup>1</sup>Department of Plant Breeding, Justus-Liebig University Giessen, Germany; <sup>2</sup>Huazhong Agricultural University, Wuhan, China Mauricio Orantes Bonilla     Mauricio.D.Orantes-Bonilla@agr.uni-giessen.de

Genetic diversity combined with adaptation and combining ability are the key prerequisites for hybrid vigour. In the narrow gene pool of rapeseed (*Brassica napus*, genome AACC), novel diversity can be introduced from related oilseed crops, for example *B. rapa* (AA) and *B. carinata* (BBCC). However, *de novo* synthesis of new-type *B. napus* is known to cause genomic rearrangements (e.g. homoeologous exchanges), which can have both negative and positive consequences on agronomic traits. The synthetic semi-winter *B. napus* accession G3D001 combines A subgenome introgressions from *B. rapa* with C subgenome introgressions from *B. carinata*. The genetic distance from natural *B. napus* combined with a good *per se* performance makes it potentially interesting for use in hybrid breeding. To understand the extent of the impact of genome structural variation (SV) in G3001, we generated a draft genome assembly as a template for SV, genomic, transcriptomic and epigenomic analysis. Long-read Oxford Nanopore Technologies (ONT) reads from a single *B. napus* G3D001 plant were assembled with Canu v1.8. The preliminary draft assembly consists of 1456 contigs, a contig-N50 of 13.9 Mbp and ca. 95% of BUSCO genes present in the eudicotyledons and embryophyta plant databases. Significant genome rearrangements were observed. Interestingly, the assembly revealed a deletion of chromosome C02 and a corresponding duplication of homoeologous chromosome A02. These and other rearrangements were confirmed by aligning G3D001 long reads to the reference genomes of the winter-type *B. napus* reference genomes Darmor v.4.1 and Express 617 (v1.0 draft reference). Express 617 x G3D001 F<sub>1</sub> hybrids were produced by crossing the plants used for assembling the parental genomes. Future studies will evaluate the effect of parental genome structural differentiation on hybrid performance. Long reads will be generated from the F<sub>1</sub> hybrids to investigate inherited patterns in the unbalanced genomes and *de novo* SV in relation to the parents, along with pattern analysis of messenger RNA, small RNA and methylated DNA from the parents and the hybrid. Overall, it is hoped that the proposed integrative *omics* approach can contribute to a molecular understanding of heterosis.

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## Can resynthesized rapeseed be genomically stable?

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Rapeseed (*Brassica napus*, AACC) is a young allotetraploid species formed by the hybridization of *Brassica rapa* (AA) and *Brassica oleracea* (CC). However, resynthesized *B. napus* lines are often unstable and infertile, unlike natural *B. napus*. Meiotic stability in natural *B. napus* may have arisen through allele inheritance from the progenitor species or via one or more *de novo* mutations post-polyploidisation. We tested these hypotheses by characterizing a diverse set of resynthesized *B. napus* lines for chromosome rearrangements, allele inheritance, fertility, and meiotic behaviour. SNP genotyping was performed using the Illumina Infinium *Brassica* 60K array, and allele copy number used to infer translocation events between the A and C genomes. Approximately 52% of lines (91/174) with SNP genotyping information were homozygous, as expected; cross-contamination resulting in heterozygosity was common in older lines maintained for many generations in the field. Self-pollinated seed-set (average 611, range 0 – 3876 per plant) and genome stability (number of copy number variants) were significantly affected by the interaction between both *B. rapa* and *B. oleracea* parental genotypes. Most lines (94%) showed evidence of unbalanced translocations between the A and the C genomes, where loss of one homoeologous region was not balanced by the presence of an extra copy of the other homoeologous region. Our results show that some resynthesized lines are more stable and fertile than others, and support the hypothesis that allelic variants inherited from parental genotypes affect genome stability in synthetic rapeseed.

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## **Quantitative blackleg resistance and gene presence-absence variation in elite *Brassica napus***

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Stem canker, caused by *Leptosphaeria maculans*, is one of the major diseases in oilseed rape worldwide. Especially in Europe, Australia and North America, it is of major economic importance. Many R genes have been described, which lead to resistance in juvenile plants. However, due to pathogen evolution, these highly effective monogenic resistances are not durable. Our aim is to identify new quantitative resistances in elite breeding material. A combination of both resistance types might allow to develop a more efficient and durable protection against *Leptosphaeria maculans*. We are investigating a multiparental mapping population derived from seven commercial varieties. A highly susceptible variety was crossed to six accessions known to carry broad quantitative resistances. In total, this population comprises 354 DH lines from six subpopulations. These 354 accessions, as well as the parents were genotyped using the *Brassica* 60K Illumina Infinium SNP array. Genome-wide association studies using Single Nucleotide Polymorphism (SNP) markers and phenotypic data from greenhouse and field trials revealed several known and new QTL regions. In addition, we included Single Nucleotide absence Polymorphism (SNaP) markers by analyzing segregation patterns of failed genotyping calls. This approach provides us with additional markers and QTL associated with presence-absence variation (PAV). To confirm the prevalence of presence-absence polymorphisms in QTL regions, we sequenced the seven parental lines using Oxford Nanopore Technologies. This long-read sequencing technology allowed us to detect short- and long-range PAVs covering gene coding sequences. Associations of these gene PAVs with blackleg resistance were subsequently evaluated. Multi-year field trials and greenhouse trial results are being used to identify environmentally stable QTL for applications in marker-assisted breeding.





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## Towards a stable and diverse Brassica hexaploid crop

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Interspecific hybridization and polyploidization processes are known to confer advantages such as hybrid vigor and increased environmental tolerances. Although cultivated diploid and allotetraploid *Brassica* species which contain different combinations of the A, B and C genomes exist, there is no naturally occurring allohexaploid that contains all three genomes (AABBCC). Despite this, there are traits in each of the *Brassica* species that, if combined, can potentially produce a new species with many advantageous features. However, there are currently three major challenges in the production of *Brassica* allohexaploids as a viable crop type: generation of sufficient genetic variability, proof of agricultural potential, and genome stability. In this study, we are focusing on improving genome stability and on increasing the genetic diversity of our material. To do this, we have combined several *Brassica* allohexaploids from different origins: *B. napus* × *B. nigra* (naponigra = A<sup>n</sup>A<sup>n</sup>B<sup>i</sup>B<sup>i</sup>C<sup>n</sup>C<sup>n</sup>), *B. carinata* × *B. rapa* (carirapa = A<sup>r</sup>A<sup>r</sup>B<sup>c</sup>B<sup>c</sup>C<sup>c</sup>C<sup>c</sup>), *B. juncea* × *B. oleracea* (junleracea = A<sup>j</sup>A<sup>j</sup>B<sup>j</sup>B<sup>j</sup>C<sup>o</sup>C<sup>o</sup>), and (*B. napus* × *B. carinata*) × *B. juncea* (NCJ = A<sup>n/j</sup>A<sup>n/j</sup>B<sup>i/c</sup>B<sup>i/c</sup>C<sup>n/c</sup>C<sup>n/c</sup>). The cross-compatibility between these species combinations and genotypes varies, with many of the potential hybrid seeds ending up as aborted embryos. One of the most fertile genotypes was an NCJ hexaploid which also showed highly regular meiosis, making it a great candidate for a genomically stable genotype. Overall, the naponigra hexaploid types were the least fertile and also produced very few seeds in combination with other genotypes. From all crossing combinations, we produced 8246 new hybrid seeds, from which we selected a subset from the best combinations based on the number of seeds obtained and the genotypic diversity. In the future, we aim to compare the new hybrids to the inbred parental lines for chromosome pairing behavior during meiosis and seed setting. At the same time, we are in the process of analyzing chromosome complements and chromosome segregation in the progeny of putatively stable, advanced-generation allohexaploid lines using high-throughput SNP genotyping via the Illumina Infinium *Brassica* 90K array. The identification of genotype- or species-specific factors related to fertility and genome stability in *Brassica* allohexaploids will be useful for producing a novel, stable crop species.

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**Genetic variation for seed protein traits in diverse *B. napus* germplasm**Isabelle Deppé<sup>1</sup>, Jasmin Vettel<sup>1</sup>, Rod Snowdon<sup>1</sup>, Benjamin Wittkop<sup>1</sup><sup>1</sup>Department of Plant Breeding, IFZ Research Centre for Biosystems, Land Use and Nutrition, Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany Isabelle Deppé    Isabelle.F.Deppe@agr.uni-giessen.de

As Europe's leading oilseed crop, oilseed rape (*Brassica napus*) complements high seed oil content and quality with a high-value protein in the meal after oil extraction. To counter dependency on soybean imports, there is a growing demand for alternative sources of vegetable protein. Seed protein from oilseed rape is considered to be of excellent quality for both animal and human nutrition and is competitive with the quality of protein gained from soybean. The quality is mainly determined by the two major storage proteins, the high molecular weight globulins (cruciferin) and the low molecular weight albumins (napin), which respectively account for around 60 and 20 % of the total seed protein. The remaining proteins consist mainly of oil body proteins (oleosins) which have only minor nutritional relevance. Studies have shown a positive correlation between nitrogen uptake and the absolute amount of seed protein within oilseed rape, making the seed protein an important sink to improve nitrogen use efficiency. Intensive breeding for low seed glucosinolate content has reduced the relative content of napin in favor of cruciferin, since seeds of low-glucosinolate varieties have a reduced sulphur availability which simultaneously down-regulates the respective biosynthetic pathways.



In this study we focus on assessing genetic variation for amino acid composition within a diverse panel of winter-type *B. napus* accessions (BnASSYST diversity panel). In addition, the ratios of storage protein fractions (cruciferin and napin) will be analysed to identify possible relationships between these two seed protein traits and genotypes with high quantities of essential amino acids independent of the cruciferin:napin ratio. Genome-wide association studies will give further insight into the genetic architecture of these important seed storage protein traits.

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## **Annual tree-ring detection and segmentation using deep learning**

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Tree-rings record annual growth in temperate trees, which reflects the response of that genotype to the experienced environment. We implement a Mask R-CNN model to detect and segment tree-ring boundaries. Pixel-wise segmentation of microscopy images of increment cores allows for automated measurement of annual growth in temperate conifers, increasing phenotyping capacity and replicability of measurements.

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**Mapping of resistance genes against *Aphanomyces euteiches* in pea**Sandra Färber<sup>1</sup>, Irina Weil<sup>2</sup>, Thomas Meyer-Lüpken<sup>2</sup>, Willem Molenaar<sup>2</sup>, Athanassios Mavridis<sup>3</sup>, Holger Budahn<sup>1</sup><sup>1</sup>Julius Kühn Institute, Institute for Breeding Research on Horticultural Crops, Erwin Baur Str. 27, 06484 Quedlinburg; <sup>2</sup>van Waveren Saaten GmbH, Auf der Feldscheide 1, 37124 Rosdorf; <sup>3</sup>Diagnostic Laboratory, Griesebachstr. 6, 37077 Göttingen Sandra Färber  sandra.farber@julius-kuehn.de

*Pisum sativum* (L.) belongs to the most important legumes worldwide. It is a valuable source for proteins and other nutrients in human nutrition and animal feed. In agriculture, peas are important crops for soil improvement because of their ability for nitrogen fixation. Besides many other diseases, the root rot complex is a major threat for legumes and leads to enormous yield and quality losses. Wide crop rotations are not enough to prevent infection. Therefore, breeding for resistant or tolerant varieties is of great importance to counteract this threat. *Aphanomyces euteiches* Drechsler is one of the main components of the root rot complex causing typical symptoms of yellowing and browning of root and shoot tissue.

The aim of this study was to identify resistance-associated markers in a mapping population of 316 F<sub>2</sub> plants generated by crossing a high-performing garden pea variety, susceptible to *A. euteiches*, with a recombinant inbred line, used as resistance donor. Fifteen descendants of each F<sub>2</sub> plant were inoculated with a defined number of *A. euteiches* oospores and were cultivated for four weeks under optimized conditions in a climatic chamber. Genotyping of the F<sub>2</sub> plants with known anchor markers from literature was followed by analysing the DNA of 203 plants with the GenoPea 13.2k microarray (Tayeh et al. 2015). After assigning 4,070 SNPs to seven linkage groups, several QTLs with a LOD > 3.8 were identified. Most of them correspond to known QTLs from literature (Desgroux et al., 2018; Hamon et al., 2013). SNPs representing the QTL regions were used to develop KASP-assays. All plants of the validation population were genotyped with these efficient markers. By calculating the effects of all markers for each genotype, a prediction of the respective resistance status was done. This prediction will now be validated by phenotyping the validation population using the same procedure as for the mapping population.

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**Towards understanding the phenological development of quinoa by expression analysis of putative flowering time genes**Nathaly Maldonado<sup>1</sup>, Dilan Sarange<sup>1</sup>, Christian Jung<sup>1</sup>, Nazgol Emrani<sup>1</sup><sup>1</sup>Plant Breeding Institute, Christian Albrechts University of Kiel

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Quinoa (*Chenopodium quinoa* Willd.) has interesting properties, such as high nutritional value and high tolerance to abiotic stresses like drought, salinity, frost and heat. The primary requirement for quinoa cultivation in Northern Europe is the day-length adaptation through modification of flowering time. Therefore, our study aims to understand the flowering time regulation in day-length-sensitive and –insensitive quinoa accessions in response to photoperiod. We analyzed the expression of candidate genes for flowering time based on the knowledge from the close relative sugar beet (*Beta vulgaris*). We performed spatial and diurnal expression analysis using Real Time Quantitative PCR (RT-qPCR) for paralogs of 10 different candidate genes in leaves of photoperiod-sensitive and –insensitive quinoa accessions under short- (8 hours light; SD) and long-day (16 hours light; LD) conditions. Our results showed striking differences in the expression profiles of the candidate genes under LD and SD. Moreover, transcripts of CqFT1 and CqFT2, homologs of the main flowering time regulators in sugar beet, were only detected under SD conditions. These results suggest an independent regulation of flowering time under different photoperiod regimes. Under SD, the expression of both CqFT1 and CqFT2 was upregulated at late developmental stages, which suggests an alternative role other than flowering time regulation for these genes in quinoa. Moreover, unlike in sugar beet, we did not observe antagonistic regulation of CqFT1 and CqFT2 in quinoa. CqCOL paralogs showed higher expression at the reproductive stage under both SD and LD conditions, which might indicate their possible role as floral integrators in quinoa. We will combine and compare the expression data with the data derived from a genome-wide association study (GWAS) for flowering time with 324 quinoa accessions to identify the putative candidate genes for flowering time in quinoa.

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## Transcriptome resources for successful breeding of non-food bioenergy crop *Silphium perfoliatum*

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Cultivation and deployment of high-biomass-yielding non-food perennials for energy production could make a contribution to the mitigation of global problems in climate change and energy security. Cup plant (*Silphium perfoliatum* L.) represents a promising alternative to silage maize as an energy crop for biogas production. The economic importance of this bioenergy crop has grown continuously within the last decade, especially in Germany, where its total acreage reached almost 2,000 ha in 2017. Cup plant is characterized by a long blooming period, and thus its cultivation may counteract the widely observed decline in insect populations.

Despite the growing interest in this crop, the existing genetic and genomic data remain scarce. Cup plant belongs to the *Asteraceae* family and is related to the common sunflower, however, it possesses a larger genome of about 8 Gb. In this study, Illumina RNA sequencing (RNA-Seq) was performed on samples from cup plant seedlings, leaves, stems, roots, rhizomes, buds, female and male flowers. For the characterization of its transcriptome, we profited from the availability of the recently sequenced genome of *Helianthus annuus* L. Tissue-specific gene expression was investigated, as was the expression of genes related to cell wall and lignin biosynthesis that could potentially influence the biogas yield. The transcriptome sequence resources will serve for molecular marker mining and help to develop innovative breeding strategies to achieve a successful cultivation of this new high-yielding perennial crop.

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## Characterizing a wild beet translocation in sugar beet conferring resistance to the beet cyst nematode

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The beet cyst nematode (BCN) *Heterodera schachtii* is a main pathogen of sugar beet (*Beta vulgaris* L.). It has a broad host range including many species from different plant families such as *Amaranthaceae* and *Brassicaceae*. After completing their life cycle in the root, the females attached to the roots form a cyst with several hundred eggs that can survive in the soil for up to ten years. Crop wild relatives have repeatedly been used as important resource for breeding disease-resistance and tolerant plants. Sugar beet lines containing a chromosomal translocation from wild beet (*P. procumbens*) have been proven resistant against BCN. Previous studies have shown that the translocation originating from chromosome 1 of *P. procumbens* is attached to chromosome 9 of sugar beet. In order to find the *Hs1-2* gene responsible for resistance against BCN, we are using whole genome and transcriptome sequencing to compare the genome of resistant and susceptible sugar beet translocation lines. During the course of this project, a detailed genomic analysis of the translocation region enabled us to find the breakpoint at which the translocation occurred. We determined its distinctive genetic features and we could hypothesize how this translocation occurred.

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## Glutathione S-transferase as a potential marker for mutation breeding in poinsettia (*Euphorbia pulcherrima* Willd. ex Klotsch)

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Poinsettia is a popular and important ornamental crop, mostly during the Christmas season, due to its range of bract colourations, which are obtained either through classical breeding (crossing) or mutagenic breeding (radiation). The success of radiation breeding in poinsettia is highly genotype-dependent, and molecular markers have not yet been described for pre-selection of promising genotypes. Therefore, we developed a PCR-based marker for an anthocyanin-related Glutathione S-transferase gene (GST) containing a highly mutable Single Sequence Repeat (SSR) locus. The coding sequence (CDS) of the GST gene was sequenced from a range of red- and white-bracted varieties of poinsettia. Moreover, the varieties were genotyped using an approach based on the fluorescent labelling of PCR fragments. The white varieties occurred in high frequency after radiation mutagenesis of the red varieties, followed by shoot development and trait selection. Thus, red and white poinsettias from the same varieties are referred to as 'pairs', due to their highly similar genetic background. The CDS sequencing showed a 4 bp indel in all white varieties, but only for a few of the red 'heterozygous' varieties and in none of the red 'homozygous' varieties. A region surrounding the 4 bp indel (~200 bp) was PCR-amplified and resolved in an acrylamide gel in order to check for allelic configurations. All white varieties showed a homozygosity status for the allele containing the 4 bp indel. In contrast, red 'heterozygous' varieties showed both copies of the allele (with and without the 4 bp indel), and red 'homozygous' varieties showed a homozygosity status for the allele without the indel. The indel is located in a SSR locus, with a trinucleotide motif (CTTC3) composition, which result in a putative early stop codon on the amino acid sequence of the GST protein. GST genes play an important role in anthocyanin transportation, since GST mutants show phenotypes with a visible lack of pigmentation, such as *bz2* from maize, *an9* from petunia, *tt19* from *Arabidopsis* and *f13* from carnation. The SSR locus in the GST gene is a potential marker to aid the radiation mutagenesis in poinsettia breeding. Moreover, the presence of the 4 bp indel in white varieties in a recessive homozygosity state might explain the phenotype, which may be due to the lack of a functional GST protein.

Keywords: Mutation breeding, marker-assisted breeding



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## Population analysis of sugar beet and wild beets

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Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is one of several cultivated beet varieties that is believed to be derived from sea beet (*Beta vulgaris* ssp. *maritima*), a wild beet species native to European coasts. We have previously generated a reference sequence of the sugar beet genome and performed comparisons to a small number of genomes of other beet genotypes and one sea beet accession. In order to chart the variability of wild and cultivated beets, we have set out to perform whole-genome sequencing of a large set of cultivated and wild beet accessions, the majority of them obtained from public seed repositories. We performed large-scale phylogeny analyses on a total of more than 600 *Beta* accessions including *B.v. vulgaris*, *B.v. maritima*, *B.v. adanensis*, *B. macrocarpa*, and *B. patula* to assess the population structure of the *Beta* genus and to find evidence for the origin of beet domestication. A read-mapping and variant calling pipeline was developed to identify variation among the accessions in relation to the sugar beet reference genome. Principal component analyses and model-based clustering approaches were applied on the variant data to further resolve the population structure of beets.

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**Quinoa sequencing and detection of haplotype blocks for genome scaffolding**Heinz Himmelbauer<sup>1</sup>, Alexandrina Bodrug<sup>1</sup>, Felix L. Wascher<sup>1</sup>, Nancy Stralis-Pavese<sup>1</sup>, Hermann Buerstmayr<sup>2</sup>, Juliane C. Dohm<sup>1</sup>

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Quinoa (*Chenopodium quinoa*) is a crop plant that has been under cultivation in Latin America for more than 7500 years. Today, quinoa is grown in an area ranging from Colombia to Chile, as well as in parts of North America, in France, and in other countries. We sequenced and analyzed about 150 quinoa accessions and performed a phylogenetic analysis. We further show how to use this resource for genome scaffolding, a long-lasting challenge when improving contiguity of non-reference genomes. Genomic regions can be characterized by a set of haplotypes in a population, and a collection of accessions is going to carry blocks of similar haplotypes throughout their genomes because of a shared ancestry. Haplotype blocks can be detected based on single nucleotide polymorphisms from variant calling datasets. Re-appearance of the same blocks in multiple regions forms the basis for scaffolding of fragmented genome assemblies. Number and similarity of haplotypes in a genomic region is dependent on the size and heterogeneity of the population used for variant calling as well as the genetic history of the crop. We developed the approach based on re-sequencing data of quinoa and *Arabidopsis* populations.

Poster 79

**Evolutionary dynamics of the repeat landscape in sugar beet and its wild relatives**Lisa Blazek<sup>1</sup>, Juliane C. Dohm<sup>1</sup>, Heinz Himmelbauer<sup>1</sup><sup>1</sup>University of Natural Resources and Life Sciences (BOKU), Department of Biotechnology, Institute of Computational Biology, Muthgasse 18, 1190 Vienna, Austria Lisa Blazek    [lisa.blazek@boku.ac.at](mailto:lisa.blazek@boku.ac.at)

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is a young crop plant that originated from wild sea beet (*Beta vulgaris* ssp. *maritima*), a coastal plant native to Western and Southern Europe. It has been shown that transposons have influence on the genome structure and gene functionality of beets. Of the many different repeats contained in a genome, only a small subset is intact and fully functional. However, there are many defective repeats which still can be mobilized by genes encoded on intact elements. Thus, the genome is constantly in motion: Transposons get inserted into new positions in the genome; thereafter, selection and mutational processes act upon them. Repeats disrupting crucial functions will disappear quickly, while other elements, which are neutral or even beneficial, will stay on. By comparing different genomic sequence data of domesticated beets and their wild relatives, we assess the mutagenic events that took place in the beet genome in the recent evolutionary past, and explore the role that transposons have played in the evolution of the beet genome. Advances in the repeat-related knowledge of the beet genome may discover new insights about recent transposon evolution and will provide a foundation for further improvements of beet as a crop plant.

Poster 81

**Fine mapping of a genomic segment associated with the traits carbon isotope composition, water use efficiency and drought sensitivity in maize (*Zea mays* L.)**Viktoriya Avramova<sup>1</sup>, Eva Bauer<sup>1</sup>, Sonja Blankenagel<sup>1</sup>, Stella Eggels<sup>1</sup>, Sebastian Urzinger<sup>1</sup>, Monika Frey<sup>1</sup>, Chris-Carolin Schön<sup>1</sup><sup>1</sup>Plant Breeding, Technical University of Munich

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High yielding crop varieties with improved water use efficiency (WUE) and drought tolerance are needed for a sustainable agriculture facing climate change. Using near isogenic lines (NILs), we showed that a single genomic segment on chromosome 7 affects several drought-related traits in maize, i.e. WUE, grain carbon isotope composition ( $\delta^{13}\text{C}$ ), leaf growth sensitivity to drought, stomatal conductance, stomatal density and leaf abscisic acid (ABA) concentration. Such a genetic co-localization of multiple traits may be due to pleiotropic effects of the same gene(s) and/or tight linkage of causal genes. To understand the genetic basis of the investigated traits, their interplay and trade-offs and to define their causal gene(s), we generated recombinants based on the NILs. The well-characterized genetic material carries overlapping segments of the target region, which enables the genetic dissection of traits in this segment. We performed extensive phenotyping of the recombinants and combined physiological (e.g.  $\delta^{13}\text{C}$ , WUE, stomatal characteristics) and metabolic (e.g. ABA) measurements, which allowed us to break down the target segment. Using bioinformatic approaches, we selected potential causal genes that may underlie the observed phenotypes and characterized them molecularly. These selected target genes will be functionally analyzed in controlled environments to validate their role for the observed traits, to test for pleiotropic effects and to study possible trade-offs in breeding.

Poster 83

## Red clover: breeding strategy based on plant phenotyping and cpDNA genotyping

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Red clover is one of the most common forage grasses and cover crops. So far, many cultivars have been bred of this species. Unfortunately, cultivars lose their distinctiveness over time due to cross-breeding.

Changes in the gene pool and genetic erosion lead to lower tolerance for winter hardiness and higher susceptibility to pests and diseases. On the other hand, there is new demand on the market for cultivars as ingredients in functional foods or for cultivation as ornamental plants. Therefore, it is important to identify new breeding material that possesses high resistance to pests and diseases, thus complementing the gene pool of red clover.

In this study, we have analysed morphological traits of wild red clover populations *ex situ* and *in situ*. It was found that there is a high variation in morphology, which might not correlate with genetic diversity due to G x E interaction. Due to this fact, a method of molecular biology was employed. We used chloroplast DNA (cpDNA), because its genome sequence has considerable variation within and among species of the *Fabaceae* family. Ten primer pairs were constructed and tested on red clover and *Trifolium medium*, a similar species from the *Fabaceae* family, as well as *Triticum aestivum* and *Lolium multiflorum* from the the *Poaceae* family, to ensure their specificity and efficiency. All of them amplified fragments in red clover cpDNA. The size of amplified bands varied from 75 bp to 150 bp. In the next step, sequencing of plants with distinctive morphological features was done by using most efficient primers. However, variation between different genotypes was low.

### Acknowledgements:

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Poster 2

**Genetic dissection of grain elements predicted by hyperspectral imaging associated with yield-related traits in a wild barley NAM population**

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As we all know, global food security is a major challenge to cope with in future. Besides the rising demand for calories, however, also the nutritional value of crops will play a key role. Low micronutrient concentrations cause the so-called hidden hunger, which is the reason of several health issues. The political will for effective change in this area was manifested in the annual International Conference on Nutrition and Growth, which led to the Rome Declaration on Nutrition in 2014 with more than 170 governments declaring a commitment to action to end all forms of malnutrition.

Therefore, we screened and evaluated the wild barley nested association mapping (NAM) population HEB-25 for nutrient concentrations with regard to grain yield to further deepen the understanding of the genetics underlying grain nutrient concentrations. For this purpose, we introduce a high-throughput method for phenotyping grain nutrient concentrations of 15 relevant elements (C, B, Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, N, Na, P, S, Zn) by hyperspectral imaging (HSI). This enables a cost-effective and fast way of determining grain nutrients without laborious and expensive lab work. We could show that HSI offers sufficient power to conduct genome-wide association studies (GWAS) on nutrient traits and discuss beneficial wild alleles as a potential source to improve grain nutrient concentrations in future breeding. In this regard, a QTL linked to *GIBBERELLIN 20 OXIDASE 2 (HvGA20ox<sub>2</sub>)* significantly increased several grain elements without yield loss.

Poster 4

## **MAGIC-EFFICIENCY: Genetic analysis of nitrogen efficiency regulation and selection of efficient winter wheat varieties from the MAGIC-WHEAT population WM-800**

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The management of nitrogen (N) fertilization has a big environmental and economic impact in modern agriculture. Therefore, the genetic dissection of nitrogen efficiency regulation offers the opportunity to contribute to climate protection. The MAGIC-EFFICIENCY project aims to investigate the genetic architecture of nitrogen efficiency in winter wheat using the WM-800 MAGIC population (Sannemann et al. 2018). Therefore, 1) main-QTL effects, detectable across both nitrogen treatments, 2) N-treatment-specific QTL effects, detectable only in one N-treatment and 3) efficiency-QTL effects, resulting in a significant higher N-efficiency between different N-treatments will be estimated. Genotypic data of WM-800 were collected through Illumina wheat 15k SNP array and Affymetrix 135k array analysis, carried out by TraitGenetics, Gatersleben. The assays delivered 27,685 informative SNPs with physical positions according to wheat Refseq 1.0 (IWGSC 2018).

The population was investigated in a first field trial at Martin Luther University Halle-Wittenberg in 2019 under two contrasting nitrogen levels (N0 – 100 kg N/ha, N1 – 240 kg N/ha) and phenotypic data for developmental and yield traits were collected, combining classical scoring and sensor-based phenotyping with UAVs (unmanned aerial vehicles). The descriptive statistics from the field trial showed significant differences with a p-Value < 0.001 between the treatments and genotypes for heading, milk ripeness, maturity, height, number of kernels per ear, TKW and yield. The field trial results will be complemented with investigations of root morphology (FZ Jülich) and high throughput phenotyping (BASF), both under two contrasting nitrogen levels.

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Poster 6

**Breeding for priming triggered leaf rust resistance in barley**Anna Marthe<sup>1</sup>, Karolin Pohl<sup>2</sup>, Nina Bziuk<sup>2</sup>, Kornelia Smalla<sup>2</sup>, Adam Schikora<sup>2</sup>, Frank Ordon<sup>1</sup>, Gwendolin Wehner<sup>1</sup><sup>1</sup>Julius Kühn-Institut (JKI), Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany; <sup>2</sup>Julius Kühn-Institut (JKI), Institute for Epidemiology and Pathogen Diagnostics, Messeweg11/12, 38104 Braunschweig, Germany Gwendolin Wehner  gwendolin.wehner@julius-kuehn.de

Leaf rust (*Puccinia hordei*) is one of the major diseases of barley (*Hordeum vulgare* L.) leading to yield losses up to 60%. Even though the resistance genes *Rph1* to *Rph26* are known, most of them have already been overcome. In this context, priming may be an opportunity to enhance resistance to *P. hordei*. Bacterial communities, such as the soil bacteria *Ensifer meliloti*, are known to induce resistance by priming. During quorum sensing in populations of Gram-negative bacteria, they produce *N*-acyl homoserine lactones (AHL), which induce resistance in plants. Therefore, the present study aims to detect genotypic differences in the response of barley to AHL, followed by the identification of genomic regions involved in priming capacity of barley. A diverse set of 200 spring barley accessions was treated with a repaired *Ensifer meliloti* natural mutant strain *expR+ch* producing a substantial amount of the AHL oxo-C14-HSL and a transformed *E. meliloti* strain carrying the lactonase gene *attM* from *Agrobacterium tumefaciens*, which inhibits AHL production. For *P. hordei* resistance the diseased leaf area and the infection type was scored 12 dpi (days post-inoculation) and the corresponding relative susceptibility was calculated. Results revealed significant effects ( $p < 0.001$ ) of the bacterial treatment indicating a positive effect of priming on resistance to *P. hordei*. Based on the observed phenotypic differences and 23,417 filtered SNPs derived from the Illumina 9k iSelect chip and genotyping by sequencing (GBS), 5 quantitative trait loci (QTL) associated to improved resistance to *P. hordei* after priming with *E. meliloti expR+*, were identified on the short arms of barley chromosomes 6H and 7H. In the next step, KASP markers will be developed, facilitating marker assisted selection of priming-efficient accessions in barley breeding. Moreover, genes in QTL regions might be interesting candidates for further research on the mechanisms of plant-microbe interactions. Furthermore, the same barley collection will be tested for priming efficiency for *Pyrenophora teres* resistance.




Poster 8

## Automated phenotyping to identify leaf and stripe rust resistances in wheat genetic resources

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Bread wheat belongs to the most important crops for human nutrition and its production needs to be increased by 60% until 2050 to ensure food security. Yearly, leaf and stripe rust infections caused by *Puccinia triticina* and *Puccinia striiformis*, respectively, result in significant quality and yield losses up to 70%. Growing of resistant cultivars carrying effective resistance genes is the most efficient and environmentally friendly solution to avoid these yield losses. However, many of the resistance genes present in cultivars were broken down after the emergence of virulent races in the past. Therefore, the identification of genetic resources with effective resistances is an important task. To achieve this, the wheat *ex-situ* collection of the IPK Gatersleben has been analyzed for rust resistance. More than 9,700 winter wheat accessions were phenotyped in greenhouse experiments by applying an automated high-throughput technology, which allowed a time-saving and precise phenotyping by digital image analysis. The system, consisting of an automated multispectral imaging system and machine learning-based software, analyzes multiple infected leaf segments per genotype and calculates the infected leaf area reliably. Data obtained from greenhouse experiments were successfully correlated to field data of a core collection of 800 genotypes. In contrast to the greenhouse experiments, rating in the field was done visually at the adult plant stage. Quantitative phenotypic information will be used in a Genome-Wide Association Study (GWAS) approach based on phenotyping and Genotyping By Sequencing (GBS) data with minimal impact of population stratification, as well as in pre-breeding and breeding programs with the final aim to develop the IPK Gatersleben's *ex-situ* wheat collection to a comprehensive breeding and research tool.

Poster 10

**Ascorbate-glutathione cycle for scavenging H<sub>2</sub>O<sub>2</sub> in bread wheat genotypes (*Triticum aestivum* L.) during drought stress and following recovery**Aydinli Lale<sup>1</sup>, Aliyeva Durna<sup>1</sup>, Huseinova Irada<sup>1</sup><sup>1</sup>Institute of Molecular Biology and Biotechnologies, Izzat Nabiyev 11, Baku AZ 1073, Azerbaijan Aydinli Lale     aydinlilale@gmail.com

Every year, agriculture faces serious losses due to the global climate change and drought. The best way to deal with drought is developing tolerant and productive varieties. Bread wheat (*Triticum aestivum* L.) genotypes (tolerant Gobustan, sensitive Tale 38) were cultivated under natural conditions. The changes in the amounts of the main components of the ascorbate glutathione cycle, ascorbic acid (AsA) and glutathione (GSH), and activities of ascorbate peroxidase (APO) and glutathione reductase (GR) were determined during the wax ripening stage in leaves of drought-exposed plants and on the 7th day after rehydration. It was established that the ascorbic acid amount increased in both genotypes under drought compared with the watered variant and then decreased after rehydration, and this decrease was more pronounced in the Gobustan genotype compared with Tale 38. Moreover, *de novo* synthesis of the glutathione molecules and glutathione amount increased at the expense of the regeneration of the oxidized form in both genotypes under drought compared with the control and after rehydration, this amount recovered relatively in the Gobustan variety, while it remained unchanged in Tale 38. Activities of APO and GR, which are the main enzymes of the ascorbate-glutathione cycle, were different in various genotypes. Thus, APO and GR activities in durum wheat genotypes increased slightly under drought, and after rewatering, these values reached the control. In contrast, the activities of these enzymes in bread wheat genotypes decreased sharply (about 2 times) and increased after rewatering. In this case, GR activity reached the control value, whereas APO activity was lower compared with the watered variant. The results clearly indicated that the ASC-GSH cycle responded differentially in drought-tolerant and drought-sensitive wheat genotypes during drought and recovery.

Poster 12

**Breeding tomatoes with improved flavour using a breeders' sensory test**

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Tomatoes are the most important vegetables in Europe and worldwide. However, consumers are often not satisfied with the quality, especially the flavour, of tomatoes. The organoleptic quality of fresh tomatoes (aroma, taste, texture) is a complex trait and difficult to assess. Modern breeding programs focus primarily on high yield, firmness and long shelf-life, whereas flavour is mostly neglected. Flavour of fresh tomatoes can be assessed either by sensory analyses or by analytic measurements.

Selection in early generations is based on a high number of genotypes, but a low number of fruits per genotype. A trained panel is not able to assess genotypes during these breeding steps. To face this constraint, we introduced the so-called "breeders' sensory test" that is suitable to evaluate a high number of small samples with a small team. To assess the potential of this test and the selection for flavour related traits in the F<sub>2</sub> generation, twelve 'quality x quality' and five 'quality x yield' crosses were produced. In 2017, the performance of ten F<sub>2</sub> plants per cross and at least four individuals of the eleven parental cultivars were grown in a low-input organic and a hydroponic conventional production system. All samples were evaluated for flavour-related traits using the breeders' sensory test (sweetness, sourness, total aroma, tomato-typical aroma) and instrumental methods (total soluble solids, titratable acids).

Correlations between corresponding sensory and analytical traits were highly significant ( $p < 0.001$ ). The correlation between sensory sweetness and total soluble solids was 0.70 for the low-input organic and 0.68 for the conventional production system. The correlation between sensory sourness and titratable acids was 0.49 and 0.64, respectively. To evaluate the efficiency of selection with the breeders' sensory test we compared coefficients of variance of F<sub>2</sub> plants and parental plants per cross. Hence, environmental (variation between genetically identical plants of both parents) and genetic (variation between F<sub>2</sub> plants minus environmental variance) variance were estimated per cross for flavour related traits. The observed coefficients of variance of F<sub>2</sub> plants exceeded the environmental variance in most cases. Heritability was estimated for the parental cultivars and ranged from 0.70 to 0.95 for sensory and analytical traits. Therefore, we conclude that selection for flavour related traits in the F<sub>2</sub> generation is expected to be successful and that the breeders' sensory test is a suitable tool to improve the flavour of tomatoes.

Poster 14

## Improving pea production – yield and nitrogen content of pea cultivars with different leaf types

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Pea (*Pisum sativum*) has a very important role for food and feed, especially in organic agriculture. There are two main plant architectures of pea i.e. normal leaf and semi leafless. The second group is based on the *AFILA* mutation (*afaf TLTL*) having a pair of stipules at each axilla and developing tendrils instead of leaflets. Semi leafless peas have advantages in standing stability and therefore, a reduction of pathogens in comparison to the normal type. However, normal leaf genotypes have advantages in light interception due to their larger foliage. Since the 1980s, pea breeding has almost focused on semi leafless cultivars.

In total, 54 pea genotypes including 24 normal leaf cultivars and 30 semi leafless cultivars from different germplasm resources were screened in three environments in Central Germany. Field trials were performed using a randomized block design in two replicates with 5m<sup>2</sup>/plot, 100 seeds/m<sup>2</sup>. The field traits were scored, light interception was measured by AccuPAR ceptometer LP80, and nitrogen content in seed and straw was analysed by Advanced Purge and Trap (APT) technology (Elementar).

The correlation between seed yield and straw yield within the normal leaf group and within the semi leafless group was positive ( $r= 0.56$  and  $0.41$ , respectively). Also, the nitrogen content in seed and straw was positively correlated within both groups of leaf-type ( $r= 0.68$  and  $0.52$ , respectively). In general, genotypes with normal leaf are lower in seed and straw yield (52.3% and 25.2%, respectively), but they have a higher nitrogen content in seed and straw (9.8% and 31.3%, respectively). The heritability for yield and nitrogen content in seed and straw was always above 0.90.



In conclusion, pea cultivars with normal leaf type are interesting parents for breeding due to their higher protein content, though in comparison, they are lower in yield.

Poster 16

## Soybean stem termination gene *Dt2* affecting agronomic characters and stress tolerance in early maturity genotypes

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In soybean, plant architecture is playing a key role in determining yield and adaptability to particular environments. Late maturity soybean cultivars have a determinate stem growth (*dt1/dt1*), in which elongation of the main stem ceases at the onset of flowering, and a terminal raceme of flowers is developed. In contrast, most early maturity soybeans have an indeterminate stem growth (*Dt1/Dt1*) with stem elongation and flowering taking place simultaneously, producing longer stems with more internodes. Recently, the locus *Dt2* has been genetically characterized, causing a terminal flower raceme and semi-determinate stem growth in a *Dt1/Dt1* background through a dominant epistatic effect of *Dt2* over the *Dt1* allele. The dominant allele for semi-determinacy (*Dt2*) is considered as a recent gain-of-function mutation and might be of interest for adapting plant architecture particularly in early maturity soybeans. For studying the *Dt2* gene effect, lines from bi-parental populations segregating for the *Dt2* vs. *dt2* phenotype were evaluated in the east of Austria (Gross Enzersdorf, Tulln; growing seasons 2014-2016, 2018-2019) for agronomic and seed characteristics. Semi-determinate (*Dt2*) stem growth was clearly recognizable at the flowering stage due to the formation of terminal flower racemes. Consequently, semi-determinate stems had larger numbers of pods towards the distal end of the stem but a lower number of total nodes than indeterminate (*dt2*) stems. Moreover, semi-determinate lines had a 10-16 cm shorter plant height, earlier maturity, lower oil and sucrose content and lower thousand-seed weight than indeterminate lines. Grain yield differences between *Dt2* and *dt2* lines were significant in most experiments. In high-yielding environments, yield in *Dt2* lines was 5-8% higher than in *dt2* lines. Contrastingly, in water-stress environments, yield of *Dt2* lines was significantly lower than for *dt2* lines. Under water stress, drought symptoms such as missing or reduced pod formation at the terminal raceme position of *Dt2* lines was visible. Moreover, hyperspectral reflectance phenotyping was suggesting differences between *Dt2* and *dt2* related to water content.

Meanwhile, the *Dt2* allele had been further characterized as a transcription factor possibly influencing stomatal density apart from other pleiotropic gene effects. This has been verified in *Dt2* lines exhibiting a larger number of stomata on leaf surface than *dt2* lines.

The present results indicate drastic effects of the *Dt2/dt2* alleles on shoot development and yield characters. However, the semi-determinate *Dt2* allele apparently is associated with increased drought sensitivity which is a serious limitation in water stress environments.

Poster 18

## Use of digital image analysis for the flower color evaluation in ornamental sunflower

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Sunflower (*Helianthus annuus* L.) is broadly used as an ornamental plant in landscape gardening, but also as a potted plant and a cut flower. Since aesthetic traits, including color, are most important for newly developed ornamental plants, sunflower petal (ray floret) color has a high value for the development of new genotypes and its position on the horticulture market. The most common methodology for the evaluation of sunflower petals is based on UPOV guidelines for sunflower. By the guidelines, the color of sunflower ray florets can be described as *yellowish white*, *light yellow*, *medium yellow*, *orange yellow*, *orange*, *purple*, *reddish brown* and *multicolored*. Although there are photographs to define these color categories, the provided material does not give clear information on the color definition, especially the *multicolored* category. The main obstacle of this methodology is its high subjectivity and necessity of high expertise for evaluators. In order to make the process of evaluation of sunflower petal color more objective, we propose a new methodology that combines image segmentation (pixel-based classification), and UPOV sunflower guidelines for the definition of color groups (classes). Images of six sunflower genotypes (Ring of Fire, CMS1-30, Heliopa, Dwarf, Neoplanta and Pacino Gold) were used in the software analysis. Visual results of this process of image segmentation presented different colors for the examined varieties. This visual presentation serves as a guideline for an evaluator to determine whether there is more than one dominant color in the examined genotypes. Of the examined genotypes, Heliopa and Pacino Gold only have one dominant color, while results of other examined genotypes show more than one dominant segmented color. The proposed method groups pixels of segmented ray florets into two dominant clusters and graphically presents the position of their mean vectors in Lab color space regarding mean vectors of UPOV color classes. Moreover, the nearest neighbor classifier is used to classify pixels into UPOV classes and the percentage of pixels belonging to each class is given. Due to the early stage of this research, there is also an opportunity for development and adaptation of the proposed image analysis methodology for similar tasks in different fields of plant research.

Poster 20

**Assessment of plant architectural traits by processing 3D scanned point clouds of *Brassica napus***Andreas Eckert<sup>1</sup>, Rod Snowdon<sup>1</sup>, Andreas Stahl<sup>1</sup><sup>1</sup>Department of Plant Breeding, Justus Liebig University

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Architectural traits have a high impact on the resource use efficiency of crop plants. In oilseed rape, for instance, the inclination of leaves along with the angle and spatial arrangement of side branches and siliques are important morphological characteristics. They are highly relevant for the efficiency of solar radiation utilization, and because of that, they are in a broader sense relevant for maximizing crop yield per unit of water and nitrogen uptake.

The architecture of rapeseed plants is highly flexible and complex. Due to the indeterminate growth of side branches and the forming of bifurcations, oilseed rape shows an extremely high compensation potential between yield components. Consequently, it is difficult to systematically dissect and quantify the complex and flexible architecture. Thereby, the manual assessment of architectural traits is extremely labor-intensive and error-prone, making it unsuitable for large-scale screenings of populations with sufficient replications. Thus, breeders are generally restricted to no more than manual ratings of the canopy architecture and canopy density in their selection decisions.

Here we present a semi-automated approach to dissect systematically architectural traits in oilseed rape. We scan full-sized plants using a 4-channel multispectral camera on the 3D PlantEye Dual-Scanner F500 (Phenospex, Heerlen, The Netherlands). The Dual-Scanner setup enables the subsequent merging of generated sensor data to a stereo vision. In this way, an accurate digital 3D-point cloud of the scan-captured plant is compiled, providing spectral and spatial information for each individual datapoint. We use this point cloud to extract phenotypic values for different architectural traits like branching behavior. For data analysis, we apply a new pipeline-approach to the 3D image data. This approach is further used to enable the description of genotype-specific plant morphology, more precisely for plants in post-flowering developmental stages.

The preliminary results show the huge potential of 3D imaging and analysis as a high throughput method in phenotyping for morphological traits of oilseed rape to dissect the response to abiotic constraints as reduced nitrogen or water availability. We expect this will give deeper insights into the relevance and genetic determinants of plant architectural traits for higher seed yield and resource efficiency.

Poster 22

**Exploring *Camelina sativa* stress tolerance mechanisms for future breeding approaches**Peter Stasnik<sup>1</sup>, Dominik Großkinsky<sup>1</sup>, Bikram Pandey<sup>1</sup>, Zoltan Takacs<sup>1</sup>, Johann Vollmann<sup>2</sup>, Claudia Jonak<sup>1</sup><sup>1</sup>Center for Health and Bioresources, AIT Austrian Institute of Technology, Tulln, Austria; <sup>2</sup>University of Natural Resources and Life Sciences (BOKU), Tulln, Austria Peter Stasnik     peter.stasnik@ait.ac.at

Plants frequently encounter adverse growth conditions. Climatic factors, such as extreme temperatures, drought and contamination of soils by high concentrations of salts, are major abiotic environmental stressors that delay growth and development and thus reduce crop yield. It is now accepted that in future, European agriculture will face severe losses in quality and quantity from abiotic stresses.

*Camelina sativa* (false flax, gold-of-pleasure) is an ancient, low-input European oil seed crop which has not yet undergone intensive breeding. *Camelina* has a high adaptability to changing environmental conditions and an inherent resilience compared to other crops that often lost plasticity and stress tolerance during domestication. Thus, *Camelina* is a promising resource to identify previously unknown tolerance mechanisms and harness them.

To study the abiotic stress tolerance of *Camelina sativa*, a set of different camelina genotypes are analyzed under high salinity conditions. This approach will be complemented by assessing the response to different water/drought regimes. Phenotypic changes, physiological parameters, as well as molecular markers will be analyzed and correlated with the stress response/tolerance of the different camelina lines. As plant growth and development, and thus the agronomic value of crops, are tightly linked to their metabolism, a focus will be laid on key carbohydrate metabolic enzymes. Furthermore, selected antioxidant enzymes will be explored for their potential to serve as breeding markers for stress tolerance in *Camelina sativa*. To do so, we recently established a platform for multiple metabolic and enzymatic measurements in a microplate format in our lab. The overall goal of the project is to decipher the stress tolerance mechanisms of *Camelina sativa* to support innovative breeding.



Poster 24

**Genetic contributions to tolerance for downy mildew pathogen *Peronospora variabilis* in a South American panel of quinoa**Carla Ximena Little<sup>1</sup>, Miguel Correa<sup>2</sup>, Karl Schmid<sup>2</sup><sup>1</sup>Department of Plant and Environmental Science, University of Copenhagen, Denmark; <sup>2</sup>Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany Karl Schmid  karl.schmid@uni-hohenheim.de

Quinoa (*Chenopodium quinoa* L.) is an ancient crop of South America and considered as a suitable crop for cultivation outside its native region because of its favorable nutritional qualities and stress tolerance. However, one limiting factor for quinoa cultivation within and outside its center of origin are fungal diseases, of which downy mildew is the most important. Therefore tolerance against fungal pathogens needs to be an important breeding goal for future breeding programs. We tested whether genetic resources differ by their degree of tolerance to the downy mildew pathogen *Peronospora variabilis* by screening a panel of 132 genotypes from South America for their tolerance against *P. variabilis* under greenhouse conditions. We used severity of infection (percentage of diseased leaf tissue) and sporulation (proportion of infected area showing signs of sporulating structures) as measure of tolerance and correlated these traits with genebank passport data, seed saponin content, and measurements of stomatal characteristics to identify correlations with disease tolerance and estimated broad-sense heritability of the disease tolerance. For this purpose, a series of three experiments with a completely randomized block design with four blocks was fitted with linear mixed models for severity and sporulation, and a generalized linear mixed model for incidence. Response to mildew infection showed a large variation between genotypes, but high genotype by experiment variances were also found. Additionally, severity and sporulation were highly correlated, demonstrating that severity is a good indicator of the overall response of quinoa to mildew infections. None of the phenotypic traits reported in the genebank passport data were correlated to the disease response. Heritability was fairly high for severity and sporulation (0.72-0.81), but low for incidence (0.40). These results indicate that resistance to *Peronospora* is mainly quantitative, although the effect of qualitative resistance genes can not be ruled out. We resequenced all 132 accessions and combined genomic and phenotypic information to conduct a genome-wide association study (GWAS) to identify genomic regions contributing to pathogen tolerance for use in future breeding programs. We will present the results of both the mixed model and GWAS analyses.

Poster 26

**Laying a cornerstone for cup plant breeding**

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Today, the demand for plant biomass is mainly covered by maize. This leads to massive monocultures and decreasing crop diversity of our landscapes. To solve these well-known problems, we have to substitute parts of the silage maize by others or new crops respectively. The cup plant (*Silphium perfoliatum* L.) is, like sunflower, a member of the *Asteraceae* and promising to keep up with the biomass yields of maize. *Silphium* is a perennial plant with deep-going root architecture, thus more resistant to climate change, and the wild plant offers a broad range of ecological benefits like a bee-friendly long flowering period. Due to low variations and small genetic distances within the European genotypes, almost no breeding attempts have been successful until today. Therefore, a plant hunting trip along the borders of the native distribution area of *S. perfoliatum* in the US was performed. A unique collection of about 40 populations, which show multiple traits we have never seen before within the European material, were discovered and established in a field trial at *Campus Klein-Altendorf*, near Bonn, Germany. For the past two years, phenotypical data were recorded and analysed regarding the variation within the native populations and comparing it to the ones of the European genotypes. Showing gapless significant differences over the whole value range of all known genotypes, the native populations enable a basis for creating a new sustainable and competitive crop out of *Silphium*.

Poster 28

## Establishment of an image-based, high-throughput phenotyping system to monitor grapevine root architecture

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The root system, its architecture and the ability for deep rooting is one of the most important plant traits with regard to local adaptation of the plant to soil characteristics and resilience towards drought stress in viticulture. Contrary to aerial plant organs that can easily be phenotyped by imaging sensors, precise phenotyping of root traits, like the total root length, the amount of lateral roots and formation of adventitious roots is a challenging task because of the hidden growth. In the present study, different root phenotyping systems were evaluated regarding throughput, precision and applicability for grapevine breeding purposes.

Therefore, we established a non-invasive and high-throughput rhizotron system in the green house. It enables image-based phenotyping of grapevine roots growing for three weeks in low-cost rhizotrons filled with dark potting soil. All rhizotrons stand in a slanted position with roots growing along a detachable lid. Removing the lid makes the root system visible and allows its examination without pulling the roots out of the soil. The approach provides standard RGB images of vegetative traits (i.e. leaf area) and grapevine roots, which were automatically analysed by the commercially available software WinRhizo.

This rhizotron system was applied to screen the F1 progenies of two different mapping populations: (1) V3125 (*Vitis vinifera* 'Trollinger' × 'Riesling') × 'Börner' (137 genotypes) and (2) 'Calardis Musqué' × 'Villard Blanc' (151 genotypes). In two seasons, both populations were tested three times with five replicates resulting in 4320 rhizotrons in total. After determining root parameters i.e. total root length, adventitious root length and lateral root length, first preliminary QTL regions were identified correlating with these root characteristics. In sum, this low-cost root phenotyping method provides objective, precise data and facilitates plant screenings and monitoring approaches with high-throughput.

Poster 30

**FHB early detection by in-field phenomics**Sara Francesconi<sup>1</sup>, Mauro Maesano<sup>2</sup>, Federico Valerio Moresi<sup>2</sup>, Antoine Harfouche<sup>2</sup>, Giorgio Mariano Balestra<sup>1</sup>

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Wheat is one of the most cultivated crops in the world. Among the different fungal diseases affecting cereals, *Fusarium* Head Blight (FHB) of wheat is one of the most devastating ones. The infection of *Fusarium* spp. varies at a regional level, depending on weather conditions and plant phenological stage. In fact, fungal growth is favored by high temperature and humidity conditions and the infection is strictly linked to wheat anthesis. FHB symptoms involve necrosis and bleaching of heads, resulting in shrivelled kernels. Plant phenotyping is the comprehensive assessment of complex traits such as growth, development, tolerance, resistance, physiology and yield. In particular, healthy plants interact with electromagnetic radiation in a different manner from that of infected plants. Thus, imaging techniques are very helpful for detecting these differences in optical properties. Plant phenotype applications also regard the use of thermal imaging, allowing to measure plant tissue surface temperatures to study plant water relations, and specifically for stomatal conductance, because a major determinant of tissue temperature is the rate of transpiration. Abiotic or biotic stresses often result in decreased rates of photosynthesis and transpiration, thus the remote sensing of tissue temperature by thermal imaging can be a reliable way to detect changes in the physiological status of plants in response to different stresses. Hence, the aim of this work was the exploration of the potential of RGB and thermal imaging for early FHB detection in the field before the appearance of symptoms. A durum wheat field (cultivar Marco Aurelio) located in Amelia (PG, Umbria Region, Italy) was used to perform phenomics trials over two years. Inside the field, a 100 m x 100 m experimental plot was divided into 16 sub-plots marked by vertical supports. For each sub-plot, spike temperature, photosynthetic efficiency and spike sampling was performed to detect FHB pathogens as ground reference parameters. RGB and thermal cameras were mounted on an Unmanned Aerial Vehicle (UAV) and three flights were performed during May-June 2019. Thermal imaging revealed temperature differences inside the sub-plots; moreover, temperature values increased with an increase of FHB incidence and severity, while infected plants showed a decrease in photosynthetic efficiency. Future perspectives will be focused on validating the temperature readings obtained by the thermal camera with ones obtained as ground measurements, confirming the results obtained with a third-year campaign and developing a robust vegetation index able to evaluate the field "health status" starting from RGB images.

Poster 32

## Genetic dissection of anther extrusion in the MAGIC-WHEAT population WM-800

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High anther extrusion (AE) is of advantage to promote cross pollination and to ensure high level of pollen availability for hybrid wheat seed production and is therefore of major interest in hybrid breeding programs. Hence, the genetic architecture of anther extrusion was studied in the multi-parental population MAGIC-WHEAT WM-800 by genome wide association studies (GWAS) with a fivefold cross-validation to identify genes involved in a non-cleistogam flowering performance. Phenotypic data for 800 genotypes were collected in two years, showing strong variation for AE between the founder as well as within the WM-800 and high heritability of 75.66%, optimal requirements for a GWAS with 27.685 SNPs, a combination of 15k illumina array and 135k Affymetrix array.

GWAS revealed Rht-B1 and Rht-D1 as the major genetic players, whereas the short straw allele results in a lower AE. The insensitivity to gibberellin acid (GA) in the mutant allele lets speculate that the reduced cell division and elongation leads to shorter anther filaments.

Besides the old acquaintances for plant height, novel genetic regions determining anther extrusion were estimated. Chromosome 1B harboured a favourable allele from "Meister" which explained 5.25% of the phenotypic variation. According to the phenotypic data, Meister is the founder with strongest anther extrusion among all founders.

Anther extrusion can as well be influenced by swelling of the lodicule, which forms at the base of the floret. Their expansion forces the lemma and palea apart, enabling the anthers to emerge. The significant SNP on chromosome 2A is localized in a genetic region with functional annotation in wheat; a plant invertase -pectin methylesterase inhibitor. These results were strengthened by the protein evidence of a pectin methyl esterase inhibitor of *Aegilops tauschii* at the same genetic region. Pectin methylesterase (PME) is the first enzyme acting on pectin, a major component of plant cell wall, and decisive in plant development. Therefore, the estimated genetic region at QTL on 2A could play an important role in plant development regarding anther extrusion. Additionally, the significant region could coincide with the genetic location of the homoeologous orthologs of the barley cleistogamy gene *Cly1*, designated as *TaAP2-A* on the long arm of chromosome 2.

Further, a GWAS for *Fusarium* susceptibility and plant height estimated favorable and unfavorable overlapping regions for the examined traits. Therefore, we postulate that the results of GWAS for AE in combination with higher *Fusarium* tolerance would improve the modern gene pool by marker assisted selection.

Poster 34

## Effectiveness of chitosan hydrochloride on organic control of Fusarium head blight of wheat

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Economically, wheat is a highly important crop, since it ranks first in world grain production and accounts for more than 20 % of total human food calories. Fusarium Head Blight (FHB) of wheat is caused by more than 16 *Fusarium* spp., but the main causal agent is *F. graminearum*. FHB symptoms involve bleaching and necrosis of heads, but FHB incidence is also highly associated with type B trichothecenes including the vomitoxin Deoxynivalenol (DON), the most common mycotoxin found as a contaminant in foods and feeds. Regarding the actual FHB control strategies, synthetic antifungal compounds belonging to the groups of imidazoles and strobilurins are extensively applied during the wheat flowering time. In fact, FHB occurrence is strictly linked to wheat anthesis, but the application of fungicides may not be fully effective as a result of wrong application time and increasing virulence of pathogens caused by climate change. Moreover, xenobiotic fungicides are extremely dangerous for humans, animals and an issue for environmental safety, thus, current research topics need to be focused on finding new natural compounds with antimicrobial and host-biostimulant properties. Chitosan is a naturally-occurring compound that has potential in plant disease control. Several studies have shown that chitosan is able to exhibit toxicity on fungal growth and development, as well as to elicit a variety of defence responses in host plants. Based on these properties, interest has been growing in using it in agricultural systems to reduce the negative impact of diseases on yield and quality of crops. The aims of this work were to investigate the antifungal and biostimulant properties of chitosan hydrochloride against *F. graminearum* and its main susceptible host, durum wheat. In particular, we tested *in vitro* chitosan efficacy to inhibit spore germination and fungal growth, as well as different mechanisms of action. Main results underlined that chitosan hydrochloride is able to inhibit fungal development and growth at low concentrations (0.5 – 0.1 %); moreover, it has shown an important biostimulant activity on kernel germination, root and coleoptile development and on Nitrogen Balance Index values. Chitosan hydrochloride mechanisms of action revealed the ability to downregulate essential genes involved in *F. graminearum* virulence and physiology and, more importantly, 13 genes involved in the trichothecene biosynthesis pathway resulted to be strongly inhibited. Future perspectives will focus on evaluating the chitosan hydrochloride *in vivo* antifungal activity, its effect on trichothecene accumulation on mature kernels and on plant defense response physiology.

Poster 36

## Challenges in converting single nucleotide polymorphisms into KASP markers in polyploid wheat

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Information on Single Nucleotide Polymorphisms (SNPs) exists for many accessions of wheat and other crops in web databases and publications. Such SNP datasets generated using different genotyping platforms, for example the 90k Illumina Infinium wheat hybridization array, can identify useful trait-associated markers for use in marker-assisted breeding. This necessitates conversion of array SNP assays into breeder-friendly SNP assay technologies like KASP (Kompetitive Allele Specific PCR) which allow for a high genotype throughput. In polyploid crops, conversion of markers into KASP assays can be challenging due to the presence of highly similar sequence regions between subgenomes. For example, we identified 15 SNPs associated with a QTL region conferring high root biomass in bread wheat. The SNPs were detected using the 90k Infinium array and are organized in two haplotype blocks on chromosome 5B (Hap-5B-RDMa and Hap-5B-RDMb). For use in marker-assisted QTL transfer, we attempted to convert these SNPs into KASP assays using different strategies requiring various complexities of data analysis. A common problem for conversion into robust predictive assays arose due to non-specific primer binding to homeologous/paralogous sequences. This resulted in limited transferability to locus-specific KASP primers via automated KASP primer design using online tools. Another common problem was primer mismatches or product size differences, leading to preferential amplification of one allele relative to another or false calling in some genotypes (revealed by subsequent Sanger sequencing). Such errors were not predicted by the Chinese Spring wheat reference genome, indicating insufficient coverage of Hap-5B-RDMa diversity by this reference for the wheat gene pool. Thus, for successful conversion of SNP array markers into robust and predictive KASP marker assays, it is recommended to compare multiple wheat genomes/assemblies along with Sanger sequencing of selected genotypes and parents of targeted breeding populations. Applying this strategy, we developed three highly reproducible, stable KASP assays, which are diagnostic for the desirable root biomass QTL haplotypes and are sufficient to differentiate between accessions with high and low root biomass. The new markers are being used for marker-assisted backcrossing to determine the impact of enhanced root biomass in cultivars with high yield potential but poor drought adaptation.

Poster 38

**Identification of common bunt resistance gene *Bt12* in wheat**Almuth Müllner<sup>1</sup>, Bobur Eshonkulov<sup>1</sup>, Julia F. Hagenguth<sup>1</sup>, Bernadette Pachler<sup>1</sup>, Maria Buerstmayr<sup>1</sup>, Sebastian Michel<sup>1</sup>, David Hole<sup>2</sup>, Herbert Huss<sup>3</sup>, Hermann Buerstmayr<sup>1</sup>

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**Key message:** The bunt resistance gene *Bt12* was mapped to chromosome 7DS of bread wheat and KASP markers suitable for MAS were developed and validated in an independent set of lines.

Common bunt, caused by *Tilletia caries* and *T. foetida*, and dwarf bunt, caused by *T. controversa*, are particularly destructive diseases of wheat grown under organic production conditions and negatively affect both grain yield and quality. Most breeding programs de-emphasized selection for bunt resistance with the introduction of effective seed treatments and genomic research of bunt resistance is far behind other traits in wheat. Thus, only few molecular markers can be used for the selection of bunt resistance in wheat. A total of 16 race specific bunt resistance genes have been proposed to date. However, only two *Bt*-genes, *Bt9* and *Bt10*, were mapped to specific chromosomal regions on the long and short arm of chromosome 6D. In the present study, a recombinant inbred line population derived from the *Bt12* bunt differential accession PI119333 and the susceptible cultivar 'Rainer' was evaluated for common bunt and dwarf bunt in artificially inoculated field trials over four growing seasons. The population was genotyped with the Illumina 15K SNP chip and the major QTL *QBt.ifa-7DS* representing *Bt12* was identified on chromosome 7DS, explaining 39% of phenotypic variation for bunt resistance. Selected SNP markers were turned into Kompetitive Allele-Specific (KASP) markers and used to validate *Bt12* in an independent set of validation lines that included the full set of winter wheat bunt differential lines. They can thus serve as user-friendly markers for deploying *Bt12* in elite breeding material.

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Poster 40

## Comparative mapping of bunt resistance QTL in wheat

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**Key message:** Different combinations of bunt resistance QTL mapping to chromosomes 1AL, 1BS, 7AL and 7DS of bread wheat mediate resistance in response to common bunt and dwarf bunt.

Closely related fungi are the causative agents of common bunt and dwarf bunt diseases of wheat: While common bunt is caused by *Tilletia caries* and *T. foetida*, dwarf bunt is caused by *T. controversa*. Both diseases negatively affect grain yield and quality and are particularly destructive under organic production conditions. Due to the close relationship of the three *Tilletia* species, the same genes are thought to mediate resistance to common bunt and dwarf bunt. We studied complex bunt resistance mediated by multiple genes in response to both diseases in bread wheat. Two recombinant inbred line populations derived from crosses of resistant cultivars ‘Blizzard’ and ‘Bonneville’ to the susceptible cultivar ‘Rainer’ were evaluated for common bunt and dwarf bunt in artificially inoculated field and greenhouse trials over two growing seasons. The populations were genotyped with the Illumina 15K SNP chip and bunt resistance QTL were mapped to chromosomes 1AL, 1BS, 7AL and 7DS. Common bunt resistance was mediated by two major QTL *QBt.ifa-1BS* and *QBt.ifa-1AL* and explained 30-35% and 20-25% of phenotypic variation. *QBt.ifa-1BS*, although of utmost importance under common bunt conditions, was not involved in the resistance response to dwarf bunt. Dwarf bunt resistance was mediated by major QTL *QBt.ifa-1A* together with minor QTL on chromosomes 7AL and 7DS, which accounted for 30% and 10-15% of phenotypic variation. Selected SNP markers associated with the major bunt resistance QTL *QBt.ifa-1AL* and *QBt.ifa-1BS* were turned into Kompetitive Allele-Specific (KASP) markers. These were used for QTL validation in an independent set of lines and will serve as breeder-friendly markers for introgression of the 1AL and 1BS QTL in elite breeding material.

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Poster 42

**Breeding oilseed rape (*Brassica napus*) with lower glucosinolate content through functional analysis and mutagenesis**Srijan Jhingan<sup>1</sup>, Hans-Joachim Harloff<sup>1</sup>, Christian Jung<sup>1</sup><sup>1</sup>Plant Breeding Institute, CAU-Kiel, Germany

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Glucosinolates (GSLs) are heterogeneous secondary metabolites specific to *Brassicales*. They are natural organic compounds containing sulfur and nitrogen that are synthesized from glucose and amino acids as precursors. Owing to their anti-nutritive and toxic properties, GSLs play a major role in defensive mechanisms against herbivory. From a diversity panel of ~120 different compounds reported from *Arabidopsis*, 15 major glucosinolate types have been identified in *B. napus* seeds. Utilization of rapeseed meal (containing ~40% protein) as animal feed is restricted due to these toxic and anti-nutritive compounds that even in canola grade oilseed rape (00) can still reach concentrations of 25 µmol/g in seeds. As part of a larger project to develop rapeseed meal as feed for farmed fish in aquaculture, we strive to reduce glucosinolate content by knock-out of genes involved in the aliphatic glucosinolate biosynthesis and transportation pathway. The project aims to identify loss of function mutations i.e. non-sense and missense mutations within GSL biosynthesis and transporter genes in an existing Express617 winter rapeseed TILLING population. This will be achieved via a conventional TILLING by DNA mismatch analysis and a novel NGS TILLING protocol. Homoeologs for two glucosinolate biosynthesis genes and one transporter gene have been identified. Expression profiles of candidate genes in seeds and leaves of winter type oilseed rape Express617 have shown differential expression during seed maturation. Two biosynthesis genes and one transporter gene with two paralogs each have been selected as candidates for TILLING. Candidate genes have been selected according to low copy numbers and tissue-specific expression analyses in leaves and seeds at 15, 25, 35 and 45 days after pollination. 35 and 43 putative functional mutations have been screened for biosynthesis genes *BnMYB28* and *BnCYP79F1*, respectively within two paralogs each. For transporter gene *BnGTR2*, 15 putative functional mutations have been screened for one paralog so far. Loss of function mutations within the candidate genes will be combined by crossing TILLING mutants and be phenotyped for aliphatic GSL content in leaves and seeds. With successful down-regulation of genes involved in the aliphatic biosynthesis pathway, a significant reduction in the aliphatic GSL profile is expected.

Poster 44

## The role of ABA-responsive element binding factors in proline biosynthesis in *Arabidopsis* and barley

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Proline is a compatible solute and accumulates under osmotic stress, regulated under both ABA-dependent and ABA-independent processes. Studies have illustrated that ABA deficient mutants showed reduced proline accumulation and *P5cs1* expression under osmotic stress. However, the transcriptional regulators mediating proline accumulation under the ABA-dependent pathway are not well understood. In the present work, we evaluated proline biosynthesis in the quadruple mutant (*abf1abf2abf3abf4*) of four ABA-responsive elements (ABRE) binding factor (ABFs) in Col-0 background. Two weeks old plants were transferred to MS medium containing 50  $\mu$ M ABA and shoot proline was measured at 24 to 96 hours of ABA application at 24 hours intervals. Col-0 showed significant upregulation of *P5cs1* and accumulated significantly more proline compared to *abf1abf2abf3abf4* upon ABA application. However, there was no difference in shoot proline content between *abf1abf2abf3abf4* and Col-0 under control conditions (transferred in MS media without ABA). Likewise, the *P5cs1* expression and shoot proline content were significantly upregulated at one, two and three hours after dehydration. We also illustrated that *abf1abf2abf3abf4* was sensitive to drought treatment. Terminal drought for one week significantly reduced fresh weight, dry weight and relative water content in *abf1abf2abf3abf4* compared to Col-0. In addition, *abf1abf2abf3abf4* showed higher membrane damage and lipid peroxidation in response to drought. The knowledge in *Arabidopsis* will help to identify the putative ABFs and to explore their role in drought adaptation and resilience breeding in crop plants. In this regard, we produced the knock-out mutants of putative barley ABFs using CRISPR-Cas9 system and are in the process of characterizing them.

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## Morphological and molecular characterization of some wild tomato genotypes

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Tomato (*Solanum lycopersicum* L.) is the greatest vegetable species of Turkey both in production and export. Parallel to intense production activities, many diseases and pests result in significant economic losses every year. In this study, tomatoes *S. habrochaites*, *S. pimpinellifolium*, *S. peruvianum*, *S. pennellii* and *S. chilense* were evaluated for the morphological characterization according to UPOV. Also using the MAS method, Tomato Yellow Leaf Curl Virus (TYLCV), Tomato Spotted Wilt (TSWV), Tomato crown and root rot (*Fusarium oxysporum* f.sp. *radicis lycopersici* = FORL), Fusarium Wilt (*Fusarium oxysporum* f.sp. *lycopersici* = FOL), Verticillium (*Verticillium* spp.) and Nematodes (*Meloidogyne incognita*) were determined for *Ty-1*, *Ty-3*, *Sw-5*, *Frl*, *I-2*, *Ve* and *Mi* genes, respectively. To develop new varieties, tomato breeding programmes have to be systematic, and the breeders need to use different genotypes in the gene pool. In this study, morphological and molecular screening of different wild species of tomatoes and their use in future breeding studies are aimed at.

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**The power of big data integration in phenotype predictions**

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Plant breeding is widely recognised as a crucial tool for addressing the effects of climate change and feeding a growing population, especially in developing countries. To meet this demand, breeders are working on developing new varieties in a very complex landscape. Plant breeding, plant genetics, and integration of new programs into existing breeding schemes presents a unique set of demands and challenges. New technologies hold promise for expediting these advances. Here we present one aspect of our phenotype prediction tool, xSeedScore, and how the use of machine learning capabilities and advanced algorithms allows for large data simulation, which is able to predict millions of progeny to improve identification of highly quantitative trait-marker correlations, identify top lines for propagation, and integrate environmental calculations to complete the full prediction cycle of GxExM. xSeedScore accelerates the genetic gain per unit of time that can be accomplished using traditional statistical genomic selection methods or breeding alone. It also demonstrates the power of large offspring simulation in machine-learning based predictions, which is needed to tackle the upcoming challenges of breeding.

Poster 50

**Towards speeding up the breeding process of the perennial cup plant (*Silphium perfoliatum* L.)**Martin Greve<sup>1</sup>, Dr. Christian Wever<sup>1</sup>, Christoph Korte<sup>1</sup>, Julian Elfers<sup>1</sup>, Ralf Pude<sup>1</sup><sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn Martin Greve     Martin.Greve@uni-bonn.de

Cup plant (*Silphium perfoliatum*) originates from North America and has a high potential for use as a biomass plant in future. Like sunflower, the plant is part of to the *Asteraceae*. *Silphium* shows a number of ecological advantages, which are characterized by a high yield of biomass, perennial growth, a strong root system for good water management and a long and bee-friendly flowering period. However, the use of the cup plant in modern agriculture is currently only possible to a limited extent. Nowadays only wild plant material is available for cultivation. Due to this, there are several challenges for breeding or rather *de novo* domestication. This is due to a large genetic diversity and causes a certain inequality in the field. In order to optimize this status and to make the use of cup plant more attractive for farmers in a modern and sustainable agriculture, varieties of this wild plant need to be elaborated and developed by breeding processes. Cup plant has a natural generation time of two years. How can the breeding process speed up? Based on a two year cycle, the breeding process of cup plant would require a lot of time; for that reason, a speed breeding process has to be developed, which will ideally reduce the generation time by half. In the first step, the natural vernalization time needs to be reduced to a minimum. Therefore, the essential duration time for vernalization will be figured out. *Silphium perfoliatum* possesses long-lasting seed maturation and strong germ inhibition. In the second step, *in vitro* embryo culture techniques should be used for shortening natural generation time. The earliest possible point in time for further development of immature embryos *in vitro* will be determined.

Poster 52

## Natural variation in seed development and germination capacities of *Crassocephalum* species and their implication for the domestication of these orphan crops

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The tetraploid *Crassocephalum crepidioides* and its diploid relative *C. rubens* are underutilized, traditional leafy vegetables and medicinal plants that are native to tropical Africa, but grow throughout tropical and sub-tropical regions of the world. Depending on the region, various common names exist and include ebolo (Nigeria), gbolo (Benin) or akogbo, which refer to both species. English names are fireweed, thickhead, and redflower ragleaf. *C. crepidioides* and *C. rubens* belong to the family *Asteraceae*, are annuals that propagate rapidly, grow well even in marginal soils and are rich in vitamins, minerals and essential oils.

Despite their value as food sources in Western and Central Africa, ebolo is not regularly cultivated, but is still mainly harvested from the wild, and thus efforts are made to promote its domestication. To contribute, we have collected different Asian and African accessions and are investigating differences in seed development and germination capacities. This showed that *C. crepidioides*, in particular Nigerian accessions, flowered later than *C. rubens*, which was correlated with prolonged vegetative growth and strongly increased biomass gains. Moreover, *C. crepidioides* formed much more seeds than *C. rubens*, which were smaller and showed increased dormancy, in particular in the dark and at lower ambient temperatures. The increased dormancy of *C. crepidioides* seeds was associated with a hypersensitivity to the plant hormone abscisic acid, which is known to promote dormancy, but also abiotic stress resistance in plants. We are currently verifying these findings and are investigating if abiotic stress resistance, in particular resistance against temperature extremes, drought and salinity, may differ between the two species and accessions. The relevance of our findings for the development of agricultural practice and the design of breeding strategies are discussed.

Poster 54

**Experimental field trial of a triple combination anti-HIV microbicide produced in rice endosperm**Amaya Blanco-Perera<sup>1</sup>, Teresa Capell<sup>1</sup>, Victoria Armario-Najera<sup>1</sup><sup>1</sup>Department of Plant Production and Forestry Science, School of Agrifood and Forestry Science and Engineering, University of Lleida-Agrotecnio Center, 25198 Lleida, Spain Victoria Armario-Najera     mvictoria.armnaj@pvcf.udl.cat

The Human immunodeficiency virus (HIV), the causal agent of AIDS, is one of the world's most serious public health challenges, particularly in developing countries. There were approximately 37.9 million people across the globe living with HIV/AIDS in 2018. The disease cannot be cured; however, it can be controlled with antiretroviral drugs. A number of molecules used in the treatment of AIDS, such as recombinant antibodies, are produced currently in microbial or mammalian cells in fermenters and require a cold chain for distribution, making the production/distribution system an expensive platform which is beyond the reach of patients in the developing world. Molecular pharming, the production of recombinant pharmaceuticals in engineered plant cells or intact plants, offers several advantages over fermenter-based production platforms. We generated transgenic rice plants accumulating the anti HIV neutralizing antibody 2G12 and two lectins, griffithsin (GFRT) and cyanovirin (CV-N). We carried out an experimental field trial with a homozygous rice line (H-136, T3 generation) which accumulated the three molecules in the endosperm. The regulatory permits for field cultivation were obtained following the local, Spanish and EU regulations. The engineered plants were indistinguishable from wild type in terms of phenotype, agronomic properties and seed yield. Biochemical analyses are ongoing to determine protein accumulation and functionality in the field-grown plants.



Poster 56

## **The ProFaba project in SusCrop (ERA-NET) started: Improving *Vicia faba* breeding practices and genotypes to promote climate-friendly and vegetable protein production in the European Union**

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Europe produces about 30 % of its consumption of plant proteins for animal feed, resulting in an annual import of about 35 million tons of soybean and soya meal. The European agricultural system is thus dramatically short in domestic protein crops, rendering the agricultural system unsustainable. In the last decades, faba bean (*Vicia faba*), a domestic, Old-World grain legume, has been grown in the EU to a much lesser extent than what would be an adequate share of the rotations and beneficial for a resilient, sustainable and climate-friendly agricultural system. The European Union is currently funding the *ProFaba* project, coordinated by the University of Aarhus, which aims to reinforce the vegetable protein production in Europe, hence to diminish our dependency from protein import, by improving faba bean. The *ProFaba* project focuses on the most important faba bean topics, including:

- (1) Disease resistance: the *ProFaba200* panel-of-inbred lines will be phenotypically and genetically studied for resistances including mRNA expression studies;
- (2) N-symbiosis: a European collection of *Rhizobium* strains from faba bean will be established. Differences in faba bean's selectivity for *Rhizobium* strains across the *ProFaba200* panel will be associated to relevant genes using GWAS.
- (3) Protein nutritional quality: The *ProFaba200* plus a panel of mutant lines will be evaluated for seed protein features. Relevant mutations will be identified using SHORE mapping.
- (4) Frost tolerance: the Göttingen Winter Bean Population (GWP), known for its high genetic resolution for frost tolerance, will be employed in addition to the *ProFaba200* panel. The faba bean 50K SNP chip will be applied for GWAS (frost tolerance and further traits). Improved faba bean cultivars will contribute to increase farmers' revenues and the sustainability of our cropping system.

Poster 58

**CRISPR-Cas-mediated genome editing for the improvement of oilseed rape**Tahmina Islam<sup>1</sup>, Hans-Joachim Harloff<sup>1</sup>, Christian Jung<sup>1</sup><sup>1</sup>Plant Breeding Institute, Kiel University Tahmina Islam     t.islam@plantbreeding.uni-liel.de

We are focusing on improving three major traits in rapeseed: improving yield by increasing the number of seed chambers in the siliques, improving silique shatter resistance and reducing glucosinolate content in the seeds. To achieve this, we need to develop new and more efficient tools for gene editing. Regarding the regulation of the number of seed chambers, 4 genes with 13 copies are involved in rapeseed, namely *BnCLV1*, *BnCLV2*, *BnCLV3* and *BnCRN*. Complete knock-out of each of those genes, as described in the literature, has so far not led to satisfying results showing either no phenotype or too many pleiotropic effects. We strive to attain better results by multiple editing in varying combinations using a polycistronic tRNA-gRNA approach with the pRGEB32 vector. Specific target sites have already been cloned into the transformation vector and *Agrobacterium*-mediated genetic transformation of rapeseed is on the way.

Regarding silique shatter resistance, we strive to compare EMS and CRISPR-Cas9 mutants in the *BnALC* genes. We are following two strategies to obtain the same mutations in the same genotype, one is a novel approach by introducing the desired mutation by CRISPR-Cas9 driven exon exchange, the other involves fast track backcrossing by genomic background selection. The backcrossing program has already started and putative target sites for exon exchange in *BnALC* have been detected. In the past, great progress has been made in reducing seed glucosinolate content, but the commercial threshold of 18  $\mu\text{mol/g}$  seed still limits the use of rapeseed meal for human and animal nutrition. By targeting a series of key biosynthesis genes, we aim at a substantial reduction of seed glucosinolates. Our focus is a knock-out of the transcription factor *BnMYB28* and the core structure synthesis gene *BnCYP79F1* using a CRISPR-Cas9 system, and currently, *Agrobacterium*-mediated transformation with specific target sites is on the way.

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## **Mutation breeding creates desired traits in African sorghum – semi-dwarf and early maturing**

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Wad Ahmed is a Sorghum variety popular among farmers in Sudan, except that it matures slightly late and is tall, making it prone to yield losses caused by terminal drought and lodging. Gamma-irradiation of seeds followed by breeding work was undertaken to quickly and cost-effectively obtain early-maturing and semi-dwarf mutants and derived populations (M<sub>6</sub>). By high-throughput short-read sequencing we compared the mutants to their progenitors and genotyped large F<sub>2</sub>-populations (M<sub>6</sub>BC<sub>1</sub>F<sub>2</sub>) for genetic mapping. We are developing molecular markers to facilitate the use of these new traits in Sorghum breeding programs in Member States.

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## Dealing with HTTP data in modern crop breeding programs

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Modern crop breeding programs are data-driven. A breeder's decisions are based on the prediction of the genotype performance from a large number of field trials. These trials should account for environmental variability of the target region, and more importantly, they should possess a high degree of accuracy. In recent years, different robotic and sensor technologies for collecting high-throughput field-based plant phenotyping (HTTP) data have been developed. Thereby, the possibility for gaining higher overall precision, as well as data and decision accuracy from crop breeding field trials was gained. Prediction of end-of-season yield and quality will become faster with the use of cameras for hyperspectral imaging, which is important for large scale producers. Comparing big sets of images generated in the field with results of classical chemical analyses serves as an advanced crop quality prediction tool for breeders. Important steps in such data analysis are calibration, noise reduction and the search for the most significant relations. Nevertheless, assessing phenotypic traits within genetic collections is made more accurate with the aid of phenotyping platforms that record plant growth from germ to seed. Like many types of phenotypic data, HTTP data collected from the images may also have some amount of unknown variability. This type of variability can introduce bias prior to integration with phenotypic and genomic data for a final prediction model. Application of statistical procedures for outlier detection and testing for normality is required, as well as visualization tools in order to attain an optimal level of data quality. Considering the volume and frequently high correlation of HTTP data, data reduction techniques and shrinkage regressions are required for an efficient selection of the most important HTTP variables for inclusion in the statistical model. Further development of new tools for HTTP data analysis is needed for big data interpretation. Choosing the appropriate statistical model should enable relevant analyses of the obtained data to breeders and provide assistance in the decision-making process during plant breeding.

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## Genomic prediction of flowering time and yield through SNP and metabolite analysis in the barley NAM population HEB-25

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Breeding for yield performance in elite barley (*Hordeum vulgare* ssp. *vulgare*) led to a reduction of biodiversity through allele erosion, the so-called genetic bottleneck effect. Consequently, further improvement of the performance of barley becomes increasingly difficult. Moreover, classical selection methods with several years of field trials are expensive.

To accelerate the breeding progress, indirect selection methods are of great importance. The most common method is the SNP based estimation of breeding values through genomic prediction (Heffner et al. 2009). A study in maize confirmed that a reliable estimation of performance with metabolite data is also possible (Riedelsheimer et al. 2012). The advantage of genomic prediction is the early estimation of traits already in seedling stage of the plant which accelerates the selection of the best plants during the breeding process.

In the current project, we simultaneously characterized the multi parental wild barley population HEB-25 with SNPs (50K SNP chip) and through metabolic profiling of 128 or 122 metabolites of one early and one late sampling date. We merged SNP, metabolite and phenotype data to alternatively predict phenotypes based on metabolites or SNPs and compared the prediction accuracy of both methods. In addition, we will associate phenotype and metabolite expression with SNP by means of genome-wide association studies (GWAS) and identify QTLs and candidate genes which control the expression.

Results for genomic prediction delivered prediction accuracies with SNP data up to 0.97 for plant height. Using metabolite data, accuracies up to 0.61 for heading were determined. Combining of SNP and metabolite data did not increase prediction accuracy compared to SNP based prediction. Metabolites collected at the early sampling date performed better than those taken from the late sampling date. In addition, first GWAS results located QTLs controlling sugar-like metabolites.

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Poster 66

## Improving and maintaining winter hardiness and frost tolerance in bread wheat by genomic selection

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Winter hardiness is a major constraint for autumn-sown crops in temperate regions, and thus an important breeding goal in the development of new winter wheat varieties. Winter hardiness is influenced by many environmental factors, rendering phenotypic selection under field conditions difficult due to irregular occurrence or absence of winter damage in field trials. On the other hand, controlled frost tolerance tests in growth chamber experiments are often costly and laborious, even with few genotypes, which makes a genomic breeding strategy for early generation selection an attractive alternative. The aims of this study were thus to compare the merit of marker-assisted selection using the major frost tolerance QTL *Fr-A2* with genomic prediction for winter hardiness and frost tolerance, and to assess the potential of combining both measures with a genomic selection index using a high-density marker map or a reduced set of pre-selected markers. Cross-validation within two training populations phenotyped for frost tolerance and winter hardiness underpinned the importance of *Fr-A2* for frost tolerance, especially when upweighting its effect in genomic prediction models, while a combined genomic selection index increased the prediction accuracy for an independent validation population in comparison to training with winter hardiness data alone. The prediction accuracy could moreover be maintained with pre-selected marker sets, which is highly relevant when employing cost-reducing fingerprinting techniques such as targeted genotyping-by-sequencing. Genomic selection thus showed large potential to improve or maintain the performance of winter wheat for these difficult, costly and laborious-to-phenotype traits.

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**Molecular-genetic analysis of FHB resistance in a CIMMYT spring wheat line**Jakob Seereiter<sup>1</sup>, Hermann Buerstmayr<sup>1</sup>, Barbara Steiner<sup>1</sup><sup>1</sup>University of Natural Resources and Life Sciences Vienna, Institute of Biotechnology in Plant Production, Department of Agrobiotechnology Tulln Jakob Seereiter     jakob.seereiter@students.boku.ac.at

Due to the risk of mycotoxin accumulation, achieving low Fusarium head blight (FHB) infection levels in the field is a significant food safety issue. Breeding and growing resistant cultivars is a key approach to reduce the potential for FHB infestations. FHB resistance is a quantitative trait; considering spring wheat, the most effective Quantitative-Trait-Loci (QTL) for FHB-resistance have been found in Chinese cultivars such as Sumai3 and Wangshuibai. Nevertheless, there is still a need to identify new sources with high levels of resistance. We crossed the resistant CIMMYT spring wheat line CMSS97M01333S-030M-81Y-010M-2M-0Y-0FGR-0Y-0FGR (6408-1) with the highly susceptible varieties Remus and Michael and developed two populations. About 180 recombinant inbred lines (RILs) of each cross were screened in two replicates for FHB field resistance under spray inoculation in 2019. Line 6408-1 was verified to have high FHB field resistance and it was shown that it is a potential candidate for being a FHB-resistance donor. Disease severity segregated among the different RILs, showing broad diversity from highly diseased to highly resistant. Furthermore, the morphological and developmental traits plant height, date of anthesis and extent of retained anthers were assessed, as these had been described to be associated with FHB disease development. In addition, DNA of the RILs has been extracted and targeted genotyping for known major FHB resistance QTL is currently underway. So far 6408-1 has been tested negative for the prominent QTL *Fhb1*. Genome-wide marker coverage and subsequent phenotyping will allow to unravel the high FHB resistance of this new source of resistance.

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## **Dissection of the cytoplasmic effects of chloroplasts and mitochondria uncovers a remarkable contribution of the chloroplast to plant reproductive traits**

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Heritable effects of the cytoplasm on agronomic and morphological traits of plants are known since the beginning of the 20th century. A prominent example is cytoplasmic male sterility (CMS), which is used for hybrid seed production. However, the effects of the cytoplasmic genotypes of plastids (chloroplasts) and mitochondria extend well beyond this reproductive trait. In grasses, the organelle genotype contributes to yield, grain quality and disease resistance.

One of the challenges while identifying organelle-encoded loci for breeding is the separation of cytoplasmic effects from other maternal ones such as imprinting, as well as the distinction of chloroplasts from mitochondria. In the genus *Oenothera* (the evening primrose), a model species for cytoplasmic genetics, a particular karyotype structure confers meiotic ring formation. This form of functional asexuality, combined with biparental chloroplast inheritance (which is the rule in the genus), allows a genetic separation of chloroplasts and mitochondria. These analyses uncover an astonishing contribution of the chloroplast and mitochondrial genome to plant architecture and development. In addition, they demonstrate a strong influence of the chloroplast to reproductive traits in analogy to CMS. This influence is overlapping with that of the mitochondria. Hormonal signaling cascades that originate in the organelles most likely confer these effects.



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**Genome-wide association studies and genomic selection for disease resistance in *Brassica napus***Iulian Gabur<sup>1</sup>, Rod J. Snowdon<sup>1</sup>, Christian Obermeier<sup>1</sup><sup>1</sup>Department of Plant Breeding, IFZ Research Centre for Biosystems, Land Use and Nutrition, Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany Iulian Gabur    iulian.gabur@agrar.uni-giessen.de

Comparative analysis of allelic diversity and genome structural variation associated with resistance factors to important fungal oilseed rape diseases was performed using Genome-Wide Association Studies (GWAS) and Genomic Selection (GS). Resistance screening to major fungal pathogens was done in greenhouse and field experiments using a *B. napus* Nested Association Mapping (NAM) panel. Phenotyping for resistance to Blackleg, *Sclerotinia* stem rot, and *Verticillium* stem striping disease was done in different locations in Germany and France. GWAS identified a large number of significant marker-trait associations and new genomic regions associated with resistance when single nucleotide absence polymorphism (SNaP) markers derived from Brassica 60K Illumina Infinium SNP genotyping array data were included in analyses. Also, GS studies including SNaP markers increased the prediction accuracies for disease resistances. Together with resequencing data from parental genotypes, these results suggest the involvement of short-, medium- and long-range presence/absence variation in disease resistance in a recent allopolyploid crop genome. These findings will allow improving future breeding efforts on *Brassica napus* and other closely related species by targeting the newly identified presence/absence alleles involved in resistance and speeding up the overall genetic gain.

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## **High-throughput phenotyping and genetic analysis to promote breeding for enhanced nitrogen use efficiency in winter oilseed rape**

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A sufficient nitrogen supply is indispensable to crop yield formation and for meeting quality standards. However, the practice of liberally applying fertilizer can impose negative effects on the environment. Surplus nitrogen can leach into the groundwater, leading to nitrate contamination, or be released into the atmosphere as the greenhouse gas nitrous oxide. Along with environmental concerns, stringent regulations exacerbate the need for efficient crop varieties that maintain high productivity under low nitrogen applications.

While juvenile oilseed rape efficiently takes up available nitrogen, maturing plants shed significant amounts of nitrogen in the form of senescent plant organs. Nitrogen in straw accounts for additional nitrogen losses at harvest. Breeding for increased nitrogen use efficiency (NUE) in oilseed rape is a key factor in enhancing the sustainability of fertilizing practices.

Breeding requires screening large crossing populations for genetic variation. Optical sensors can rapidly and precisely phenotype large numbers of plants. Multispectral sensors capture canopy light reflectance at specific wavelengths, which are indicative of several physiological parameters of plants including their nitrogen status.

Employing a quadcopter drone, we periodically captured multispectral imagery of eight winter oilseed rape varieties grown under five different nitrogen fertilization levels. We collected plant samples throughout plant development and at harvest. These samples were subjected to destructive analyses to record morphological parameters related to plant nitrogen status. By modelling the relationship between spectral indices, plant architecture and nitrogen content, we derived algorithms to translate sensor information into nitrogen status.

This high-throughput methodology is used to phenotype a multi-parental mapping population, generated from crosses between five maternal lines distinguished for high NUE with a common paternal line featuring high nitrogen uptake, in field trials at six locations across Germany over two growing seasons. Multispectral images are periodically captured to record phenotypic data at key developmental stages. Coupled with genome-wide marker data of the entire population, we strive to model genotypic performance under low nitrogen fertilization in order to ultimately refine genetic prediction models for yield formation under low N.

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## **kWIP: the k-mer weighted inner product, a *de novo* estimator of genetic Similarity**

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Rapidly estimating genetic relatedness of individuals (or “samples”) directly from sequencing data is often desired, e.g., to establish sample identity and detect mix-up, to uncover non-obvious genomic variation, or to assess population structure without a reference genome and the associated biases. Determining genetic relatedness directly from high-throughput DNA sequencing data, quickly and in an unbiased manner, demands novel, computationally efficient methods.

We present the k-mer Weighted Inner Product (kWIP), an assembly-, and alignment-free estimator of genetic similarity along with ready-to-use software. kWIP combines a probabilistic data structure with a novel metric, the weighted inner product (WIP), to efficiently calculate pairwise similarity between sequencing runs from their k-mer counts weighted by their information content. The kWIP software produces a distance matrix, which can be further analysed and visualised. Our method does not require prior knowledge of the underlying genomes. kWIP is written in C++, licensed under the GNU GPL, and is available from <https://github.com/kdmurray91/kwip>.

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## On the first step of tropical tulip breeding in a hot and humid country such as Thailand

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In the past, no one believed that Thailand had the ability to produce tulip bulbs because there was no cold winter or a long chilling period. In fact, tulips have been planted in Thailand for more than twenty years. Some Thai companies planted tulips to show their beautiful flowers as a tourist attraction in the northern region of Thailand. However, only few researchers have thought about a breeding program for the entire life cycle of tulips in Thailand. Recently, a Thai pioneer researcher carried out tulip experiments over more than ten years. Although hundreds of tulip varieties have been planted in Thailand, there are only 5-6 varieties able to produce bulbs in Thailand such as 'Strong gold', 'Negrita', 'Ile de France', 'Golden parade' and 'Strong love'.

In the 2017-2018 season, four varieties of tulip, 'Strong gold', 'Negrita', 'Ile de France' and 'Golden parade' were planted at the Queen Sirikit Botanical Garden (Chiang Mai) and the Doi Phamon High Land Extension and Development Centre (Chiang Rai). Only the varieties which grew in Chiang Rai were able to produce bulbs with rates of 34.13%, 20.17%, 10.30% and 15.83%, respectively. This is the first reported endeavor to produce tulip bulbs in Thailand. In the 2018-2019 season, five varieties, 'Strong gold', 'Negrita', 'Ile de France', 'Golden parade' and 'Strong love', were planted in three locations: Doi Phamon High Land Extension and Development Centre (Chiang Rai), Phufa Development Centre (Nan) and Tak Horticulture Research Centre (Tak). In Chiang Rai, tulip bulbs which were ordered from the Netherlands, as well as bulbs harvested during the last year in Thailand both flowered and produced tulip bulbs. Note that bulbs collected during the last year produced their bulbs significantly higher than the newly ordered ones. In Nan and Tak, all varieties produced less flowers than in Chiang Rai, which was caused by late planting and hot weather. However, except for 'Strong love', all varieties in Tak produced bulbs with rates of 18.54%, 56.14%, 12.00% and 3.31%, respectively, while all varieties planted in Nan failed to produce bulbs. Although there were only 5-6 varieties which were able to produce bulbs in Thailand, their bulbs were selected as parent tropical tulip lines for a breeding program in the future. Conventional breeding is suitable to be done in parallel to mutation breeding, until we succeed to change tulip to tropical varieties as Thai ancestors have done previously with rose, chrysanthemum etc.

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## Different approaches to genomic prediction model validation in soybean

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When dealing with the improvement of quantitative inherited traits, such as yield, it is a promising strategy to simultaneously use genome-wide molecular markers that are able to capture all small effect loci influencing a trait. The success of implementing genomic prediction in the process of soybean breeding is determined by the ability of the developed model to predict or estimate the genetic potential of new breeding lines for a specific trait.

Cross-validation is a commonly performed validation procedure. However, in cross-validation, both the training and validation sets are tested under the same environmental conditions, which is not a realistic scenario in applied breeding and can lead to an overestimation of model performance. Therefore, besides evaluation of the effect of varying factors influencing prediction model performance, we shall examine the properties of the genomic prediction model in the external validation, analyzing the power of prediction when the genotypes of the validation set are not part of the training population, and when the validation population is tested under different environmental conditions, simulating the real breeding process.

The training population consisted of 227 diverse soybean lines that were used for the development of a genomic prediction model. The training population was evaluated for yield in three consecutive years and SNP data was obtained by a Genotyping-by-sequencing protocol. Prediction ability was evaluated using six mathematical models, including parametric and non-parametric, and was validated on two different levels: cross-validation (5-fold) and external validation (historical data).

Overall, genomic prediction ability for soybean yield was relatively high (0.60) and the results indicate a modest influence of the mathematical model and marker number on the prediction ability using cross-validation and external validation. However, the model had a varying ability to predict phenotypic performance in separate environments, with especially high prediction ability in years not impacted by yield-limiting factors, when the genetic potential was fully achieved. Improvement of model performance in cross-validation and external validation was achieved by increasing the phenotyping intensity that must reflect the target environment variability.

Obtained results indicate that genomic prediction can be integrated as part of the breeding process as useful tool that can increase breeding efficiency and decrease breeding time. Particular implementations are diverse, from germplasm screening and parental choice to forward breeding and direct selection based on genomic prediction.

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## **Perspectives on genomic selection and marker-assisted selection of malt quality traits in winter barley**

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The genetic value of untested lines in a breeding program can be predicted by using genome-wide marker data for genomic prediction. Genomic prediction is typically verified by a cross validation system resembling a selection within breeding cycles and has mainly been carried out on yield-related traits, which are available almost immediately after harvest. Some quality traits, like malt quality, are often not possible to measure in the limited time between harvest and sowing in a winter barley breeding program. The timing of data availability makes it difficult to utilize genomic selection within breeding cycles for malt quality traits. This study investigates if information from previous breeding cycles could be useful for genomic selection in this scenario. Malt quality is a complex trait consisting of a combination of sub-traits. This study focuses on three malt quality sub-traits:  $\beta$ -glucan content,  $\alpha$ - and  $\beta$ -amylase activity. The possibilities to use a reduced marker set for genomic selection on these traits with markers selected based on a mapping approach is investigated. The analysis is based on malt quality data from elite malt varieties and malt lines in the breeding pipeline of a commercial winter barley breeding program.

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## Optimizing the construction of haplotype blocks to increase genomic prediction accuracy across maize landraces

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Maize is one of the most studied crops for genomic analysis. Genomic prediction (GP) has become an important tool in plant breeding, allowing faster breeding cycles and reducing systematic phenotyping. In this genome-wide approach, the high dimensional parameter space leads to challenges in computational time and statistical analyses, demanding the development of efficient algorithms and statistical models. In this study, GP was done using SNPs and haplotype block libraries with the genomic best linear unbiased prediction (GBLUP) model for five traits assessing the early growth and development in doubled haploid (DH) lines across two European maize landraces. The main scope of this study was to optimize the creation of haplotype blocks in order to increase prediction accuracy (PA) across maize landraces.

The construction of genomic relationship matrices, the training of the model and prediction were conducted using the Synbreed package in R. The HaploBlocker package in R constructs haplotype blocks from a single nucleotide polymorphism (SNP) database, by identifying allelic sequences of arbitrary length which are shared by multiple individuals. Thus, HaploBlocker focuses on subgroup-specific linkage instead of LD as proposed by other approaches. HaploBlocker reduces dimensionality by clustering SNP windows of a given length and grouping them according to their joint allelic sequence into haplotype blocks, and finally filtering for the most relevant blocks. Haplotype blocks can be used to estimate the relationship between individuals. They might capture ancestral information better than SNPs, thus, might lead to higher PA across landraces. By modifying several parameters of the HaploBlocker package, the structure of the haplotype block library was affected in terms of number of blocks, average block length, Nind per block and coverage of the initial SNP based genotype matrix.

This study indicated that by optimizing the settings for the construction of haplotype blocks, PA can be increased. Therefore, using haplotype blocks for GP is promising for maize landraces, since the maximum absolute gain on PA over SNPs was 0.23 for the trait Root lodging (RL). Nevertheless, the small sample sizes in plant studies compared to human or animal studies are still a limiting factor for identifying clear patterns for parameter optimization. Further research is required comparing additional methods of haplotype block construction and statistical models in the context of GP across plant populations.

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**Plant height and heading date as covariates to predict Fusarium head blight in durum wheat**Jose Moreno-Amores<sup>1</sup>, Sebastian Michel<sup>1</sup>, Thomas Miedaner<sup>2</sup>, C. Friedrich Longin<sup>2</sup>, Hermann Buerstmayr<sup>1</sup><sup>1</sup>Department of Agrobiotechnology IFA Tulln University of Natural Resources and Life Sciences Vienna BOKU; <sup>2</sup>State Plant Breeding Institute, University of Hohenheim Fruwirthstr 21 70599 Stuttgart, Germany Jose Esteban Moreno Amores     jose.moreno-amores@boku.ac.at

Fusarium head blight FHB severely affects durum wheat and frequently an unfavorable correlation with morpho-agronomical traits like plant height and heading date. In this study, we assessed the prediction ability of the genomic predictions (GP) for FHB severity relying on genomic best linear unbiased prediction (GBLUP) models in a diverse panel of 178 durum wheat lines evaluated across five environments. The three approaches to include heading date (HD) and plant height (PH) as covariates into the analysis were: (i) correcting FHB severity scores, (ii) multi-trait GP alternatives, and (iii) application of restriction indexes. Models that weighted the predicted FHBs scores by restriction indexes and the models that predicted corrected FHBs values were efficient alternatives in lessening the HD trade-off with large reductions in prediction ability though. With a simulated genomic selection, HD as fixed effect in the GP model were the most suitable alternative to select a higher proportion of lines being both moderately resistant and lower-than-average flowering. Hence, an appropriate GP model given unfavorable association between traits should combine high predictabilities and adequate reduction of undesired trade-offs.

## Reference

Moreno-Amores, J., Michel, S., Miedaner T, et al. *Euphytica* 216, 22 (2020). doi: 10.1007/s10681-019-2551-x



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## Developing image analysis for phenotyping quinoa-downy mildew pathobiome

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Quinoa (*Chenopodium quinoa*), is a pseudocereal crop with favourable nutritional properties and evaluated for cultivation in numerous countries. Fungal diseases like downy mildew limit grain yield and the development of resistant varieties is therefore a central goal of quinoa breeding. Downy mildew resistance is a polygenic trait with significant genotype by environment interactions. In response to infection, a defence reaction is triggered that includes hypersensitive cell death localized at the area of attack and suppressing sporulation. On the contrary a susceptible genotype would develop lesions and sporulation under and sometimes on the lesion itself.

With the purpose of phenotyping for severity and sporulation caused by downy mildew on quinoa leaves, we developed an image analysis protocol using the multispectral and fluorescence approach.

First, we phenotyped a panel of 106 genotypes from South America by screening for their tolerance against *P. variabilis* under greenhouse conditions. We used severity of infection (percentage of diseased leaf tissue) on the adaxial side of the leaf and sporulation (proportion of infected area showing sporulation) on abaxial side. We scored 12 plants per genotype distributed in 12 blocks and three different experiments. These visual scores were analysed with a linear mixed model which gave an average of severity.

For image analysis, we selected two resistant, intermediate and susceptible genotypes respectively. We collected one representative leaf coming from a plant of each 4 blocks, repetition 1 and 2 (8 leaves). We compared the visual phenotypical average of severity (described above) and sporulation with those obtained with the imaging system (VideometerLab 4). The imaging contains reflectance in 10 bands, from UVA to NIR while the fluorescence is captured with narrow-band filters. The results were similar for diseased leaf tissue and sporulation, but also insightful at exposing the lesion area.

Phenotyping responses to downy mildew in quinoa has proven difficult for standard RGB imaging because of the interference caused by various shades of green and pink colors that the different quinoa genotypes express on their leaves. Therefore, this novel approach could resolve the problem, eliminate operator bias and give an insight on the infection process.

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**Fine-mapping of two bi-parental crosses to zoom into the genomic vicinity of the major QTL for very low vicine & convicine seed content in faba bean (*Vicia faba* L.)**

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Faba bean (*Vicia faba* L.) is a protein-rich feed and food. Major drawbacks are antinutritive seed compounds, such as vicine and convicine (VC; pyrimidine glycosides). One major VC QTL was recently mapped (Khazaei et al. 2015). The VC-pathway is yet unknown. As mapping population two F<sub>2</sub> families (two times 1000 individuals) were employed. Parents were two pairs of near-isogenic lines (Mél\*ILB; NPZlow\*NPZhigh). Genotyping in F<sub>2</sub> with candidate SNPs resulted in the requested map fragments and ultimately in highly informative DNA-markers. Phenotyping of marker-selected F<sub>2</sub> individuals (two times 100; Sixdenier et al. 1996) and employment of phenotypic data on VC allowed QTL fine-mapping.

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