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KEYNOTE: Lessons learned from decades of environmental biosafety research on the assessment and regulation of genetically modified plants

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This presentation discusses key lessons learned from biosafety research and from the regulation of “classical” genetically modified (GM) plants, and discussed how these insights can inform the assessment, management, and regulation of plants with novel traits, including those developed using New Genomic Techniques (NGT).

GM plants expressing foreign DNA (“transgenes”) have been released into the environment since the late 1980s. The most widely commercialized traits are tolerance to broad-spectrum herbicides (HT crops) and insect resistance through the expression of *Bacillus thuringinensis* (Bt) proteins. All jurisdictions regulate GM plants and – unless exempted – require a case-specific environmental risk assessment (ERA). A central regulatory principle is that ERAs must consider the specific plant, the introduced trait, and the receiving environment.

A scientifically robust ERA starts with explicit problem formulation (Devos et al. 2019). This includes identifying relevant protection goals (e.g., biodiversity), outlining plausible pathways to harm, formulating testable risk hypotheses, identifying existing evidence, and defining additional data needs to test those hypotheses. This structured approach ensures that potential harms are clearly defined, that risk hypotheses align with policy objectives, and that only relevant data are collected.

The requirement for case-specific ERAs has stimulated extensive biosafety research. Key questions include: which arthropods dominate agricultural landscapes; which organisms are most likely to be sensitive to plant-produced insecticidal proteins; and which species are most likely to be exposed. In parallel, validated methods have been developed to assess the direct and indirect, acute and chronic effects of dietary plant toxins on non-target organisms.

More than three decades of research show that genetic modification *per se* does not pose inherent environmental risks; risk depends on the expressed trait and associated management practices. Bt plants provide highly target-specific pest control and have not been shown to cause unacceptable harm to non-target organisms (Pellegrino et al. 2018, Romeis et al. 2019; Meissle et al. 2022). By reducing reliance on chemical insecticides, they have contributed to more sustainable agriculture. HT plants have transformed weed-management, offering cost and environmental benefits such as enabling reduced tillage (Macdonald et al. 2011). At the same time, concerns have been raised about the evolution of herbicide-resistant weeds (Heap 2014). Their full potential for ecological intensification, however, remains underutilized (Dewar et al. 2003). Importantly, transgenic and non-transgenic HT plants do not differ in their environmental impact.

Despite major scientific advances, particularly with NGTs, the regulatory framework for genetic engineering has changed little over the past 30 years and has become increasingly misaligned with actual risks. The EU is currently addressing this (Kahrmann & Leggewie 2024), but the legal proposal represents a political compromise among various stakeholders.

In our view, future reforms should go further: plants whose DNA has been modified without introducing foreign DNA should generally not fall under genetic engineering law. This includes classical breeding and undirected mutagenesis – already exempted from current GMO regulation – but should also cover targeted mutagenesis via NGT, provided no foreign DNA is stably integrated.

Many NGT-derived modifications can occur naturally or can be produced with methods that have a long “history of safe use”. Therefore, specific GMO regulation should only be applied for transgenic plants

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PREBreed – Pangenome Resource-Enhanced Breeding research in barley

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The PREBreed project is a highly interdisciplinary effort, bringing together partners from academia and industry in the area of genetics, (functional) genomics, modeling, bioinformatics, data management, physiology and breeding, to substantiate previously generated pangenome resources of barley with deep functional annotation, in order to gain a systematic understanding of how complex gene regulatory pathways are involved in environmental adaptation. The project builds extensively on earlier BMBF-funded research of the cooperation partners (BARLEX, TRITEX, SHAPE-P1-P3, EPIC-p-epBAR) and extends the previously established barley genomic resources to link complex genomic with functional diversity.

PREBreed is working towards the following goals: 1) Transcript evidence-based de novo annotation of 65 pangenome assemblies will give unparalleled access to the gene complement of domesticated barley. 2) Haplotype-specific annotation of cis-regulatory elements by MOA-seq in pangenome genotypes and corresponding F1 hybrids will utilize natural diversity in regulatory elements of the barley pangenome. Together with AI-assisted genome-wide prediction of the regulatory sequences of the barley pangenome, trained on pre-existing and newly generated pangenomic, -epigenomic, -transcriptomic and -cistromic datasets, we will unlock previously inaccessible information for more targeted selection in breeding and crop improvement. 3) We showcase exemplarily the need and current potential/limitation of high-throughput (HT) functional analysis of complex gene regulatory pathways by studying 400 NaN3 mutants (coding and/or regulatory sequences), contributed from an existing FIND-IT mutant population in the pangenome cultivar 'RGT Planet', in targets of barley phytohormone and root-specific gene networks. 4) For systematic testing of functional variation in root-specific gene expression and gene-regulatory networks, reporter and sensor lines are being established. 5) Systematic barley root phenotyping is used to reveal pangenome diversity of phenotypic plasticity as a proxy for new breeding targets for improved environmental adaptation and sustainable agriculture (e.g. carbon capture potential, nutrient and water use potential and efficiency). 6) Pangenomic variation in Nitrogen Use Efficiency (NUE) correlated to major plant architecture traits will link barley diversity to breeding for sustainability and resource efficiency. Differentiating populations will be used in collaboration with breeding industry for QTL detection and for the selection of breeding material as a new resource for variety improvement towards funding period 2. 7) PREBreed links to the Module C project PROGRESS, establishing new approaches for more precise and more efficient genome editing in barley.

PREBreed is an ambitious, timely and essential attempt of exploiting pangenome diversity for moving from single gene towards gene network-based regulatory network analysis of adaptive traits as a foundation for establishing innovation for future crop improvement.

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PROGRESS – Novel approaches for more precision and efficiency in genome editing of crop plants

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Genome Editing by Cas endonucleases has become a widely used approach for both, functional genetics and breeding. It allows for site-directed mutagenesis at precisely targeted genomic positions. Despite its broad application and great achievements, genome editing remains limited in its ability to reliably predict DNA repair outcomes and efficiency when it comes to very specific genetic targets. PROGRESS aims to address these limitations.

To develop a novel approach for precise genome editing, PROGRESS attempts to employ an alternative DNA repair mechanism that is highly active in plant cells, namely microhomology-mediated end joining (MMEJ). By MMEJ, DNA breaks are repaired using small homologous sequences (2-25 bp) in contrast to long homology arms (>100 bp), as in HDR. To activate the MMEJ repair mechanism on purpose, five fusions of Cas9 and different exonucleases were established and tested on four target motifs for their capability to efficiently generate the 3' single-stranded DNA ends required and to reduce repair by the predominant non-homologous end joining (NHEJ) pathway.

To enhance the efficiency of editing approaches, we target the second component of the Cas endonuclease system, the guide RNA (gRNA). Its secondary structure, formed by base pairing within the RNA strand, is crucial for the interaction with the Cas enzyme and with the targeted DNA strand. However, the 20 bp target specific part can strongly influence the formation of the proper structures by interfering with the intramolecular base pairing which destabilizes the hair pins or the overall structure of the gRNA scaffold. Therefore, we want to stabilise the scaffold by sequence modifications to enhance proper folding or minimize misfolding. In total, five new gRNA scaffolds where tested so far in comparison to the wild-type and a previously established improved scaffold. As next step, we aim to develop tailored gRNA scaffolds by single base modifications that avoid interactions with the sequence-specific part for very individual genetic targets.

The new approaches are developed along specific target genes that are provided by project partners of the PREBreed consortium. By precise editing of these genes, we aim to enhance nitrogen use efficiency and root architectural traits in barley.

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The pan-epigenome of barley reveals epigenetic consequences of structural variations

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Recent barley pangenome analyses have revealed extensive presence/absence variations and other structural variations. However, interpreting the functional consequences of this diversity requires detailed insights into the distribution of genes, regulatory sequences and epigenetic mechanisms. To address this, we constructed a barley pan-epigenome that integrates data on DNA methylation, histone modifications, chromatin accessibility, and chromatin interaction data. The resulting pan-methylome revealed high levels of DNA methylation, particularly in the CpG context, consistent with the high proportion of transposable elements in the barley genome. We identified differentially methylated orthologs across 20 diverse barley genotypes, and the methylation profiles were associated with chromatin accessibility and histone modification marks. All datasets are being integrated for functional annotation of the barley-pangenome through genome segmentation. Our comparative epigenomic analyses also uncovered haplotype-specific impacts of structural variants. For example, we found that: i) a large chromosomal inversion in the cultivar RGT Plant, while not altering overall chromatin states, disrupted local chromatin interactions near the breakpoint, potentially affecting nearby genes; ii) an inserted regulatory element at the Srh1 locus in cultivar Barke exhibited tissue-specific chromatin accessibility patterns, supposedly affecting the regulation of trichome development; iii) VRN2 deletions in spring barleys were linked to the epigenetic activation of VRN1 in unvernalized seedlings. This comprehensive pan-epigenome resource will provide a foundation for both fundamental and applied research in barley and cereal genomics.

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Data-driven and genome-edited breeding of locally-adapted wheat varieties to enhance agricultural biodiversity, sustainable climate resilience, and resource efficiency (DRIVE)

Jochen C. Reif[1], DRIVE Consortium[2]

Data science enhances many economic and scientific activities. However, plant breeding has not yet been one of them. This is due to the fact that crop scientists lack large enough curated, machine-readable datasets for artificial intelligence (AI) to analyze. The DRIVE project is developing a unique global data trust platform for breeding using wheat as a model. The platform will curate and analyze big data to identify contrasting genotypes and environments, with a focus on genotype-environment interactions. The platform will be populated with extensive machine-readable genomic, phenotypic, and environmental data from seven breeding programs. This data will be used to create new subroutines in process-based plant growth models, enabling the prediction of the plant's genomic and phenotypic responses to historic and future climatic extremes, such as drought and heat stress. The models' results will be integrated into genome-wide prediction approaches. Artificial intelligence methods will predict genotype-environment interactions, enabling the breeding of locally adapted wheat varieties. The models will be tested for their ability to predict plant performance under new environmental conditions anticipated due to climate change. The advanced phenotyping infrastructure at IPK Gatersleben, the PhenoSphere, makes this possible by simulating current and future environments under field-like conditions. To identify valuable, underutilized variations for wheat breeding, modern genome editing techniques will be used to transfer specific alleles, particularly those for new, thoroughly validated resistance genes that may have been lost during the breeding process.

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Harnessing the unexplored diversity of rye by genome-based breeding for climate-resilient grain production

The RYE-HUB consortium

D. Siekmann[1]

Since its launch, RYE-HUB has evolved from a conceptual initiative into an integrated genomic and breeding platform for rye. While hybrid breeding has successfully increased and stabilized rye production on finite arable land without increasing water and fertilizer inputs, the genomic system integration required for predictive breeding in this outcrossing small-grain cereal remains underdeveloped.

To address the limited representation of rye diversity in existing cereal genome assemblies, RYE-HUB is establishing the first rye pan-genome within a unified coordinate framework. This infrastructure consolidates sequence diversity across heterotic pools and links genomic variation to phenotypic performance, enabling cross-study comparability and long-term breeding integration.

At the core of RYE-HUB is JOSY (JKI Open-Source Rye), a multi-parental mapping population derived from the sequenced elite inbred line Lo7 and 68 gametes captured from genetically diverse European germplasm. High-density genotyping of 5,489 JOSY lines establishes a permanent community resource for dissecting Mendelian and complex traits in rye.

Research activities are structured along three interconnected innovation axes:

(i) architectural re-design of rye through the GA-sensitive dwarfing gene Ddw1 as a central innovation driver,
(ii) to unlock the agronomic and adaptive potential of semi-dwarf plant architecture, and
(iii) breeding acceleration via AI-assisted double haploid development and genome-based selection strategies.

By integrating genomic diversity, high-resolution phenotyping, and breeding informatics, RYE-HUB is transitioning from population analysis toward predictive system breeding. The project provides the scientific foundation for climate-resilient, site-adapted semi-dwarf hybrid rye and thereby contributes to stabilizing cereal production systems in Europe under increasing climatic volatility and geopolitical uncertainty.

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

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RYESILIENCE – Unravelling Drought Tolerance Mechanisms of Winter Rye for Climate-Resilient Agriculture

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RYESILIENCE investigates the impact of the GA-sensitive semi-dwarfing gene *Ddw1* on drought stress response in winter rye. Rye is known for its extensive root system; therefore, this project began by assessing the effects of drought stress on the roots of rye seedlings carrying the *Ddw1* gene compared to their tall near-isogenic hybrids (NIHs).

Root-system traits were measured in a controlled mini-rhizotron experiment [1] using six winter rye genotypes (three semi-dwarf *Ddw1* and three tall NIHs provided by HYBRO). Drought stress was induced by withholding irrigation for five days after the first leaf unfolded, and plants were harvested ten days after planting. No statistically significant differences were observed between semi-dwarf genotypes and their tall NIHs in total root length, root biomass, and number of root tips. The findings indicate similar belowground development in both semi-dwarf and tall genotypes, suggesting that the *Ddw1* gene does not impair early root growth under drought. In contrast, semi-dwarf genotypes produced significantly lower shoot biomass than the tall NIHs, resulting an increased root-to-shoot ratio independent of drought treatment.

To extend these findings beyond the seedling stage, the experiment is being repeated using larger rhizotrons, followed by pot experiments with soil to assess root development and biomass allocation under drought in a physically structured growth medium. In addition to controlled experiments, field trials have been established to relate Rhizotron and pot observations to agronomic performance under drought. The same winter rye genotypes are grown under defined drought intensities using rain-out shelters at the Julius Kühn-Institut, allowing direct comparison between controlled and field-based drought responses.

Furthermore, a mixed cropping system with semi-dwarf and tall winter rye genotypes and spring pea have been established to evaluate genotype performance under interspecific competition. These trials are being conducted at two conventional low-input sites and one organic site at the Julius Kühn-Institut and the University of Rostock. The experiments are ongoing, and data collection will be completed next year.

Overall, RYESILIENCE is progressing from conceptual and methodological development toward experimentally grounded, breeding-relevant evidence.

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RaPEQ – Rapeseed as a domestic protein source of excellent quality for human consumption

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The demand for plant-based protein as a food source for humans is constantly growing and represents a megatrend with a major impact on the food industry. The reasons for this are health, animal welfare, the environment, and climate protection. Rapeseed *(Brassica napus* L.*)* is not only the most important domestic oil plant in Germany but also has a potential of 1.12 million tons of crude protein per year, making it the most productive domestic protein plant. Rapeseed protein (RSP) has an excellent amino acid composition and a high nutritional value but is still waiting to be used more widely for human nutrition. To promote the use of RSP in the diet, the main objective of the RaPEQ project is to make RSP accessible for human nutrition by focusing on (i) improving protein yield and (ii) reducing off-taste. To achieve these goals, we focus on breeding, molecular sensory science, and molecular biology approaches. Such a comprehensive redesign of this oil plant requires a long-term strategy for scientific research and the translation of the results into breeding.

A main restriction against the use of rapeseed protein as food/ ingredient is its bitter-astringent off-taste. Using the sensomics approach, the RaPEQ project has identified nine kaempferol derivatives and, in particular K3OSS (kaempferol 3-O-(2‴-O-sinapoyl)-β-sophoroside) as the main cause for this off-taste. Quantitation by UPLC-MS/MS of these compounds in rapeseeds/ canola seeds and their corresponding protein isolates (150 samples) showed that most of these compounds exceeded dose over threshold (DoT) factors of one and, therefore, contributed to the bitter-astringent off-taste in the selected protein isolates. In addition, an increase of the major bitter compound K3OSS was observed during industrial protein production (apart from enrichment), which allowed the identification of its possible precursor as kaempferol 3-O-(2‴-O-sinapoyl-β-D-sophoroside)-7-O-β-D-glucopyranoside. These results may contribute to the production of less bitter and astringent rapeseed protein isolates by optimizing breeding and downstream post-harvest processing.

Consequently, we aim to reduce or eliminate these bitter-astringent tasting compounds. Technological approaches as e.g. masking and enzymatic degradation are pursued as well as on the biological side classical breeding methods, genome-wide approaches and, finally, the identification and use of putative knock out mutants for key genes of flavonol biosynthesis leading to kaempferol. We were able to identify two knock-out mutants of flavonol synthase genes (FLS1-1, FLS1-2) which in combined homozygous double mutant plants lead to a reduction of K3OSS below analytical detection limit. To quickly introduce these mutants into elite rapeseed lines, a novel “High-Speed Breeding” method has been established. “High Speed Breeding” combines “speed breeding” with a novel strategy of “fast-track backcrossing”. Aiming to improve protein yield and predicting the content of the bitter off taste kaempferol derivatives, we apply and further refine genomic and phenomics selection models.

The current results and achievements of RaPEQ phase 3 will be presented, as well as an outlook on further planned work.

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Epigenetic control mechanisms of cold-induced flower induction and flower bud development in Brassica napus and Arabis alpina

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We are studying the epigenetic regulation of the flowering response to winter cold in *Brassica napus* and *Arabis alpina*. In Arabidopsis the well-characterized FLC gene encodes a MADS-domain transcription factor that represses the transition from the vegetative to flowering state, and its expression is repressed by exposure to cold through accumulation and spreading of repressive histone modifications. However, under field conditions, several crucifers, including *Brassica napus*, have been shown to initiate flowering and produce floral buds in autumn, which open only several months later in spring. How floral bud development is regulated by cold and whether FLC gene regulation by histone modifications is important in this process is unknown. We did a functional analysis of all 9 *FLC* homologs in rapeseed, while in its diploid relative Arabis alpina we functionally analysed downstream genes of the *FLC* ortholog PEP1 and the paralogous MAF genes. In rapeseed all FLC homologs were mutated by a CRISPR-Cas9 approach in the spring type Westar and the winter type Express617. In *Arabis alpina* the *MAF* cluster of tandem duplicated genes was mutated in the reference Spanish accession using a similar CRISPR-Cas9 approach, and *PEP1* was mutated in Scandinavian accessions in which cold-treatment regulates floral bud development. We have examined the impact of single and multiple mutations on flowering time and vernalization requirements in both species. Furthermore, we identified genome-wide effects of different cold treatments on gene expression and histone modifications in wildtype plants and mutants.

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Quinoa for future diversified agricultural systems (Q4F)

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The minor crop quinoa *(Chenopodium quinoa)*
Minor crops like quinoa have a great potential to increase the resilience of future agricultural systems and contribute to agrobiodiversity. Quinoa produces a highly nutritious grain and shows high stress tolerance. A market and a value chain already exists for this crop in Germany, but production based on available varieties is not very competitive because of its short breeding history.

Objectives
The project Q4F aims at alleviating this situation for quinoa by (i) mapping relevant agronomic and resistance genes, (ii) Germany-wide modeling of quinoa production and developing an ideotype for cultivation in Germany, and (iii) creating genomic resources including genotyping arrays for marker-assisted selection and genomic prediction to facilitate the rapid breeding of new varieties. These objectives will be achieved by extensive phenotyping of experiments in the laboratory, greenhouse and field trials using image analysis; the use of whole genome, targeted long read and transcriptome sequencing; and the use of crop models and models for genomic prediction. Q4F will use diverse genetic material that includes several hundred genbank accessions reflecting native diversity, progeny of >10 crosses of diverse parents and ca. 15 European quinoa varieties in the different work packages.

Morphological traits (WP1)
Work packages include the characterization of germination and early vigor traits in controlled conditions (Rakasi et al. 2026) and morphological traits (e.g., branching, panicle shape, anther extrusion) in field trials with and without competition by neighboring plants to evaluate the phenotypic plasticity and its effect on yield components induced.

Disease resistance (WP2 and WP3)
A detached leaf assay screening for resistance against the fungal pathogen *Sclerotinia sclerotiorum* and transcriptome analyses; screening of resistance against *S. sclerotiorum* under field conditions.

Resistance against herbivory (WP4)
Screening against herbivory by multiple insect species will be carried out under controlled and field conditions, as well as an investigation of the role of epidermal bladder cells (WP4).

Genetics of traits and crop models (WP5 and WP6)
Phenotypic data will be combined with existing genome resequencing data to analyze their genetic architecture using GWAS, QTL mapping and differential gene expression. A complementary analysis of past and new field trials will parametrize a crop model and estimate genotype x environment x management (GxExM) interactions, simulations of crop rotations with quinoa and define a quinoa ideotype for current and future climates as breeding goals. Phenotypes, crop models and ideotypes will be synthesized and genetically linked using genomic prediction. Genotyping arrays for marker-assisted selection and genomic prediction will be developed based on this model.

This work will lay the foundation for finding key traits and genes that ensure high and stable yield in variable environments and contribute to agrobiodiversity for future characterization and utilization in genomics-based quinoa breeding to develop new quinoa varieties that are of interest and economic value for the private plant breeding and agricultural sector in Germany.

Partners
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Optimizing water use efficiency in hops *(Humulus lupulus* L.*)* for a sustainable production HOPTIMIZE

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The HOPTIMIZE project accelerates hop *(Humulus lupulus* L.*)* breeding for climate resilience and end-use quality by integrating genomic prediction, functional genetics and targeted phenotyping. Funded under PLANT2030, the consortium unites the Bavarian State Research Center for Agriculture (hop breeding), the Chairs of Plant Breeding and Brewing & Beverage Technology at TUM, the Agricultural Technology Centre Augustenberg and the Hop Marketing Cooperative (HVG e.G.). Its three complementary objectives are: (1) develop genomic prediction models for yield stability and water use efficiency to speed up breeding success; (2) identify and functionally characterize genes controlling yield stability, water use efficiency and hop quality, including allele diversity and effect sizes; and (3) define breeding targets for cone secondary metabolites tailored to various industrial applications.

A multiparental population segregating for drought and heat adaptation was generated and clonally propagated to enable replicated field trials across three locations in 2025. Nearly 350 female full- and half-sib progenies plus diverse genetic resources were planted in an augmented design that balances spatial constraints and experimental error control. Favorable growing conditions and intensive management supported optimal plant development. Developmental, fitness and quality trait measurements on first-year plants were taken to evaluate their suitability for genomic selection.

Molecular characterization of the multiparental population combines genotyping-by-sequencing (GBS) and with a custom SNP array, leveraging GBS marker density and array robustness. Because the US ‘Cascade’ reference genome poorly represents European germplasm, we are constructing a genetic map to integrate SNPs from the two genotyping platforms.

From 2026 on, two locations will receive contrasting water regimes—simulated drought focused on flowering (based on historical stress patterns) versus near-optimal irrigation—to capture genotype × environment interactions and drought stress performance. Phenotyping encompasses developmental, yield and physiological traits (including field water use efficiency) and chemical profiling of cone bitter acids, aroma compounds and polyphenols. Two hundred genotypes will be used for pilot production of standardized non-alcoholic beers, assessed by sensory panels to link cone chemistry with sensory perception and to test for trade-offs between drought tolerance and brewing quality.

Analytically, genomic selection models will predict breeding values at the seedling stage to enable earlier selection for complex traits, while GWAS for oligogenic traits will identify causal regions and markers for marker-assisted selection. HOPTIMIZE aims to deliver tools and targets for efficient, quality-aware breeding to safeguard the sustainability and competitiveness of German hop production under climate change.

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ViLHair – Vitis Leaf Hair – A new functional trait for sustainable and climate-adapted viticulture

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Grapevines are challenged by various biotic and abiotic stresses that are exacerbated by climate change. In particular downy mildew (DM), caused by *Plasmopara viticola*, can lead to significant crop losses if it is not controlled with regular plant protection measures. Traditional breeding of new grapevine varieties has made great progress in the development of adaptive traits and thus making an important contribution to sustainable viticulture. A number of genetic resistances to DM have been identified and introgressed as single or stacked loci into new varieties. However, the occurrence of resistance-breaking strains of P. viticola has been increasingly observed in recent years.

Ribbon trichomes can form a dense layer of epidermal leaf hairs on the underside of leaves of certain wild Vitis species. This hydrophobic physical barrier is less wettable by water and can prevent infection by retaining the germinating spores of P. *viticola* bevor they can penetrate the leaves through the stomata. Due to these features, ribbon trichomes can be considered as a first line of defense, acting upstream and complementary to genetic resistance factors. A DM resilience conveyed by ribbon trichomes has the advantage that it cannot be broken by the pathogen. Therefore, it is particularly durable and should be made genetically accessible for grapevine breeding.

To achieve this goal, as a first step F1 populations (hairy x hairless) are used for genetic mapping for genome regions associated with ribbon trichome formation. The genomes of interesting parental genotypes will be sequenced and candidate genes are being searched within the mapped genomic regions. As a next step, promising genes will be edited in order to restore the hairiness trait in an almost hairless grapevine variety. For this functional study, an innovative CRISPR/Cas gene-editing strategy is being established for grapevines, in which a modified CRISPR/Cas construct is transferred from grafted rootstock to the scion. The universally applicable method allows the generation of transgene-free grapevines with specifically altered traits, facilitating the 'Breeding by Design' approach.

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KEYNOTE: Understanding Barriers Limiting the Use of Hybridization in Crop Improvement

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Interspecific hybridization is a basic evolutionary mechanism occurring spontaneously and repeatedly during the formation of new taxa. This phenomenon is common in plant speciation, and contributes significantly to plant diversity and adaptation. Many crop species are of hybrid origin, either obviously, as wheat and rapeseed, or almost imperceptibly, as maize.

Beside natural processes, the breeders use controlled interspecific hybridization to enrich the gene pool of the most important and productive crops, which have lost much of their genetic diversity during the breeding of elite cultivars and have thus become less resilient to biotic and abiotic stress. Beyond the use of crop wild relatives, even entirely new hybrid species have been created, such as *Triticale* and *Festulolium*.

However, attempts to introgress desirable traits or increase diversity through interspecific hybridization frequently fail. This reflects the fact that many reproductive isolation mechanisms have not yet been fully described or understood. Furthermore, even if a suitable hybrid individual is successfully created, long-term benefits are not guaranteed. Alterations in genomic/chromosomal composition can result in the loss of the pertinent locus, or the underlying genes may become silenced in subsequent generations.

We aim to address some of the post-zygotic hybridization barriers limiting development and use of plant hybrids. We capitalize on our research center's long-standing experience in chromosomal genomics and the analysis of polyploid and hybrid genomes. Concerning reproductive isolation during hybridization, our focus is on understanding the mechanisms that lead to the disruption of genomic and/or transcriptional balance during endosperm development. We have discovered numerous imprinted genes in barley that are only expressed from the maternal or paternal copy at specific developmental stages. These genes are the prime candidates for the regulatory factors of this fundamental process.

Another key aspect we explore is transcriptional and genomic dominance in newly formed hybrid individuals. We have demonstrated that the ryegrass *(Lolium)* genome exhibits dominance over the fescue *(Festuca)* genome at the transcriptome level in Festulolium. Furthermore, the chromosomal shift towards the ryegrass parent is evident in several subsequent generations following hybridization. The molecular mechanism underlying the latter phenomenon involves the silencing of fescue-specific copies of kinetochore genes in the hybrid. Understanding these and similar molecular patterns in hybrids can contribute to the greater utilization of crop wild relatives for improvement of modern cultivars.

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MAZE – Accessing the genomic and functional diversity of maize to improve quantitative traits

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Genetic improvement is essential to secure sustainable crop production. Future crops will have to combine high yield potential with major sustainability factors such as stress tolerance and resource efficiency. The project MAZE has developed solutions to access native diversity of plant genetic resources in a targeted way with the goal to improve quantitative traits relevant for crop production.

MAZE has generated an unprecedented body of genomic, genetic and phenotypic data to utilize natural variation present in European maize flint and dent germplasm. For the flint pool, a large library of landrace derived doubled-haploid (DH) lines evaluated in multi-environment field trials and phenotyping platforms has been made publicly available. The DH lines were characterized based on 600k SNPs and whole genome long- and short-read sequences. A transcriptome atlas comprising a combination of 30 tissues and developmental stages as well as single root cell transcriptomes in stress and control conditions complements these data. The available comprehensive resources form an excellent basis for gene annotation, gene discovery and advancement of genomic prediction models in European maize material.

For the dent pool, we developed an 8-parent MAGIC population comprising 500 DH-lines. The eight founders were characterized with high coverage whole genome sequencing (~50x). With ~4M high-quality SNPs identified in the DH-lines (shallow sequencing, ~5X), we reconstructed the genetic mosaic of the founders in the MAGIC population using different haplotype construction methods. Field evaluations revealed significant genotypic variation for important agronomic traits, including grain yield, and drought-related traits. UAV-based phenotyping experiments were conducted to develop image-based traits for maize drought response. Precise phenotypic and high-resolution molecular data on founders and DH lines make the MAGIC population a powerful resource for gene discovery. Several QTL for yield and associated proxy traits have been identified.

In the flint DH library, we identified novel beneficial haplotypes with high potential for elite germplasm improvement. In many genomic regions, trait associations could be fine mapped down to a few Mb. Cell type-specific analyses revealed epidermis-specific transcriptomic responses to cold stress of root hair-related genes. Fine mapping of a genomic region associated with lateral root length has yielded promising results for understanding the role of root architecture under different stress scenarios. In several genomic regions, we discovered and functionally characterized candidate genes controlling photosynthesis-related traits affecting early maize growth.

We established five genome-based rapid cycling selection experiments derived from two landraces with target traits early development and testcross yield. For one landrace, DH lines derived from three selection cycles (C1, C2, C3) were evaluated together with the original DH population (C0) in large field trials in 2022-2024 for many agronomic traits. Based on the experimental results delivered by MAZE we are now able to design the bridging process that guides integration of landrace-derived material into elite germplasm with a focus on the genetic improvement of quantitative traits.

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INTEGRA – Implementing Novel Technologies to Enhance Genetic Gain towards Resilient Rapeseed

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Project aims and goals

The yield stability and further increases in productivity and quality of winter oilseed rape (WOSR), the most important oilseed crop in Central Europe, are affected by the increasing frequency and severity of extreme weather events and the changing pressure from pathogens and pests. Complex genotype-environment (G*E) interactions have to be considered in the generation of climate- and site-adapted varieties with novel traits and high productivity has to be ensured despite reduced use of fertilisers and pesticides. To address these challenges, INTEGRA develops effective new strategies for rapid identification or generation of favourable genetic variants and their combination in superior genotypes that are adapted to multiple biotic and abiotic stress factors and that are simultaneously optimised for quality traits. The pursued holistic approach integrates the development of breeding material, genomic-phenomic performance prediction, systems biology studies, and targeted genetic modification strategies for the improvement of specific traits. Multidimensional breeding and informatics technologies are utilised and two innovative R&D approaches are combined into an integrated strategy: I) genomic-phenomic prediction of hybrid performance considering G*E interactions using extensive data sets from previous work of the applicants as well as new data from newly developed inbred/hybrid varieties and II) the generation of new genetic variants in elite backgrounds by TILLING and editing of key genes for the expression of new target traits.

Project concept and implementation

The focus of the work is on stress responses in the pre-winter and post-winter growth phases, which are the most critical phases in the establishment of WOSR stands and yield development with strong G*E interactions. Knowledge gained on environmental factors and molecular/physiological mechanisms that influence and control dynamic plant performance under relevant environmental conditions will be used for targeted crossing and selection programmes that maximise the overall breeding value of parental lines and hybrids. Envirotyping is used for environmental trait detection and environments will be clustered into multi/mega environments (MET) to train G*E-derived predictions that translate plant physiological and molecular responses to relevant stress factors as indicators of plant adaptive mechanisms and response variability. In parallel to conductance of large multi-season and multi-location field trials, cultivation of selected genotypes under fully controlled, field-like environmental conditions enables the simulation of future climate scenarios and will provide data for the omics-based identification of traits associated with WOSR performance under stress. INTEGRA's integrative data science approach orchestrates data from previous projects and project data flows towards AI-ready, integrated FAIR Digital Objects (FDOs). Genomic deep learning models (DeepCRE & Enformer) will be trained and used to link the pangenome sequence with these multi-omic data and identify key regulatory gene variants associated with traits of interest. The model predictions will support prioritization and targeted de novo generation of these variants in the elite backgrounds. The associated junior research group AIM4GEM will contribute novel hierarchical modelling approaches that will improve prediction methods for accelerated breeding of climate- and site-adapted OSR varieties. Finally, the potential socio-economic and environmental impact of the progress made will be assessed through a prospective life cycle sustainability assessment.

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AIM4GEM – Strategic Mating under GxE and Phenomic Selection in Rapeseed

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Increasing climatic variability exposes rapeseed production to fluctuating nitrogen availability and water limitation, leading to pronounced genotype × environment × management interactions (G×E×M). A central objective of AIM4GEM is to improve the quantitative understanding of these interaction patterns and to translate this knowledge into operative breeding decisions. Utilizing multi-environment trial data from large rapeseed breeding populations, we characterize interaction structures within the target population of environments (TPE) and derive genotype-specific response profiles across contrasting environments. Building on this information, we investigate genomic mate allocation strategies that explicitly account for environmental response patterns. In simulations, our results indicate that conventional stability-based indices, while useful for selection, are not well suited for mate allocation. We therefore propose an alternative crossing strategy (dCross) that pairs parents with complementary interaction profiles to assemble favourable alleles across the TPE. Simulation studies suggest that when stability of final products is targeted, such strategic cross design can outperform selection based solely on mean performance or conventional stability indices. This perspective implies that stability may be more effectively generated at the cross-design stage rather than by selecting uniformly stable parents during population improvement and recombining them. These methodological developments, together with approaches for multi-trait mate allocation optimization, are implemented in the R package CrossingTools, which provides a flexible framework for evaluating and optimizing crosses under diverse breeding schemes.

In parallel, the project evaluates alternative prediction strategies based on phenomic information. We assess phenomic prediction using near-infrared spectroscopy (NIRS), routinely collected for seed quality assessment, as well as UAV-based field phenotyping, with respect to their ability to capture G×E-related variation. Our analyses show that broad-sense wavelength heritability alone does not adequately reflect predictive relevance, as strong collinearity among wavelengths allows accurate prediction using only a small subset of spectral variables (~1%). For complex traits such as seed yield, predictions may partly rely on correlated molecular components (e.g. oil or protein content), potentially leading to indirect selection responses. Traits lacking such molecular correlations, such as flowering time, appear less affected. Moreover, spectra collected across different environments exhibit environment-specific patterns, highlighting the importance of representative sampling across the TPE.

Taken together, the results contribute to a more differentiated understanding of how G×E×M can be quantified, predicted, and ultimately utilized in rapeseed breeding, supporting more informed decisions under increasingly variable environmental conditions.

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FABALOUS – Faba bean abiotic stress tolerance for improved yield stability

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Among the cold-adapted legumes, faba bean *(Vicia faba)* has the highest yield potential while producing seeds with high protein content and quality, and it leaves a significant nitrogen (N) surplus in the soil. However, biotic and abiotic stresses reduce both yield and interannual yield stability. Abiotic stress is particularly harmful for faba bean yield stability during reproductive stages, which is expected to occur more often under current climate change prospects. However, faba bean breeding is lagging behind other main crops due to different factors, among which its low multiplication rate, large genome and complex pollination system. In this situation, strengthening the current breeding activity is strictly needed to deliver more resilient faba bean cultivars. FABALOUS aims to improve yield stability under combined environmental stresses as an essential trait to turn faba bean into a productive crop for farmers.

We focus on understanding combined stress responses in the present breeding pool, which serves as a stepping stone to widening the genetic diversity and improving combined stress responses. The project is structured by three main lines of research, and first results show the potential of the expected data set.

The first line of research uses a representative set of genetically and physiologically contrasting accessions which will be analysed for the response of key agronomic and physiological parameters under single and combined abiotic stresses. These analyses include the analyses of beneficiary microbes as stress-protective biologicals. Paralleled by metabolomics, transcriptomics and epigenomic analyses, the aim is to identify gene candidates for genetic engineering that can improve vegetative and reproductive development under stress using systems biology and genome editing. Our first results show remarkable variation particularly in root architecture and early stomatal regulation under heat and combined stress. At the same time, first T0 mutants for the candidate drought tolerance gene *ADF* were generated by electric current-mediated gene editing, showing that DNA-free genome editing is a functional tool for future candidate gene evaluation in faba bean.

Second, FABALOUS combines association mapping using both a multi-parent population and a diversity set with genome structure analyses to obtain markers associated with stress resilience under very different environmental conditions. Due to the low multiplication rate in faba bean, considerable effort is necessary to produce populations of suitable size and structure. Currently, we prepare the second multiplication for the diversity set as well as the second selfing of the multi-parental population First characterizations of the diversity set are underway.

In the third line, FABALOUS benefits from access to a unique, above-ground heat pumping facility under an arable field in Bad Nauheim to study heat stress effects at field scale. Establishing this ‘heat field’ system is critical for studying genotype x environment x management effects of faba bean in crop rotations along with N fixation effects under heat stress on field scale. After sowing the pre-crop in fall, we are now installing soil sensors and optimizing UAV based phenotyping for the upcoming three vegetation periods, delivering highly valuable data on the influence of heat under field conditions.

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BeetAdapt – Adapting sugar beet for climate resilience

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Agriculture in Germany and Europe faces major challenges, including climate change and increasing sustainability requirements. Innovative technologies are needed to accelerate the development of climate-resilient crop varieties. Sugar beet is a versatile plant that can be processed into sugar, bioethanol, fertilizer, and animal feed. In Europe, approximately 197 million tonnes of sugar beet were produced in 2024 (for comparison: Germany 37 Mt, worldwide 306 Mt; FAO Statistics). However, sugar beet breeding and cultivation are demanding due to long generation times, a biennial life cycle, pronounced self-incompatibility, and complex selection processes.

BeetAdapt aims to advance sugar beet breeding methodologies to accelerate genetic improvement. The project targets key biotic and abiotic challenges including nematodes, SBR, drought stress, and bolting tendency. The interdisciplinary project addresses two highly promising research areas for the plant breeding sector, with three project partners collaborating on each topic. One team focuses on developing efficient transformation and genome editing systems. A second team works on drone-based field phenotyping, automated image analysis, and the development of autonomous drone platforms. In addition, field data and NIR seed measurements are used for phenomic selection analyses.

Current efforts in sugar beet genome editing focus on target sequence analyses and the preparation of RNPs and plasmids to validate BMC nuclease activity in tobacco and sugar beet. For phenotyping, field data acquisition and NIR seed analysis were conducted during the 2025 season, and initial prediction scenarios for genotypic values were developed using phenomic selection. Current achievements will be presented, along with an outlook on planned future work.

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Buckwheat Improvement by Modern Technologies for the Establishment of a Dual-Use Crop (BIMOTEC)

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The pseudocereal buckwheat *(Fagopyrum esculentum Moench)* was a staple food in Germany until several decades ago, when it was replaced by higher-yielding cereals. It produces a range of phytochemicals found not only in the grains, but also in the residual biomass, namely in leaves, stems and husks. Buckwheat is especially known for its high content of rutin, which is of interest for the pharmaceutical industry due to its health-promoting properties. Nowadays, buckwheat is regaining importance as gluten-free and nutritious wheat alternative and must be imported. To re-establish buckwheat production in Germany, breeding of high-yielding cultivars adapted to current climate conditions must be initiated. In line with the National Bioeconomy strategy to optimize the utilization of agricultural side-streams, BIMOTEC evaluates buckwheat's potential as multi-purpose crop. In addition to the use of grains for food, utilization of the residual biomass for the extraction of biobased compounds is investigated, such as extraction of valuable phytochemicals from leaves and husks and lignocellulose from stems. In an interdisciplinary approach, partners of the project are working on different aspects to bring forward breeding and production of buckwheat in Germany.

In the first year of the project, the Institute for Plant Sciences (IBG-2), and Institute for Bioinformatics (IBG-4) from Forschungszentrum Jülich GmbH have conducted drought experiments with fifty buckwheat cultivars from 20 different countries, to characterize the genotypic variation in drought tolerance and associated phenotypic traits. Leaf material sampled from these buckwheat cultivars under control and drought conditions were analyzed by Fraunhofer IME by comprehensive GC-MS and LC-MS analyses. This in-depth metabolite profiling facilitates the discovery of valuable secondary metabolites in the residual biomass of buckwheat. The industrial partner Phytowelt focused on establishing suitable methods for the biotransformation, extraction and measurement of myricetin from buckwheat-derived quercetin using improved cytochrome P450 monooxygenase enzymes. University of Hohenheim has performed a field trial with three genotypes at two sites with contrasting soil water availability and gathered further data for agronomic modelling from on-farm samplings. At IPK Gatersleben, genome editing technologies for the biotechnological improvement of buckwheat are developed to adapt agronomically important traits, laying the foundation for future modern breeding initiatives. As a precondition for efficient genome editing, our methods for the formation of adventitious shoots in vitro and genetic transformation are currently being optimized.

The collaborative efforts of the BIMOTEC partners contribute to re-establish the regional production of buckwheat, support German plant breeders to revive breeding on this neglected crop and give rise to novel value chains for bio-based compounds from agricultural side-streams.

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Res4StRes – Novel resources for resistance breeding against insect pests and heat stress under low cropping intensities in oilseed rape

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Insect damage, heat stress and sulfur deficiency are current threats to rapeseed production in Germany. In this project, we aim to identify and characterize biotic and abiotic resistances by developing and applying innovative phenotyping, genomic, metabolomic pathway analysis and bioinformatic approaches to systematically exploit existing and novel biodiversity for integrative rapeseed crop improvement. We are focusing on five insect pests that cause major losses in oilseed rape production in Germany and are spreading due to climate change, including the cabbage stem flea beetle (Psylliodes chrysocephala), the cabbage root fly *(Delia radicum)*, the pollen beetle (Brassicogethes aeneus), the green peach aphid *(Myzus persicae)* and the rape winter stem weevil *(Ceutorhynchus picitarsis)*. Novel phenotyping resources useful for commercial breeding will be established also by identification of metabolites co-varying with insect resistances. Germplasm will be screened using bioinformatics-assisted genetic mapping approaches to determine candidate loci. Identified candidate genotypes showing insect resistances will also be evaluated for their performance under heat stress and sulfur deficiency, to select lines with broad spectrum future breeding potential.

In the first project year five field trials with 300 plots each for insect resistance screening and three field trials for sulfur efficiency use with 400 plots and about 280 diverse genotypes each were established in seven locations throughout Germany. A total of about 10,000 pictures were produced from these locations at two time-points in early plant development with leaf damage caused by adult cabbage stem flea beetle feeding at five locations. Pictures have been visually analyzed and genotypes with similar rankings at multiple locations have been identified. A subset of one hundred genotypes has been selected and bioassays for the cabbage stem flea beetle and the cabbage root fly have been established and optimized. Bioassay screening based on activity tracking on leaf discs for the green peach aphid has been initiated. For sustainable research data management, a dedicated web-based platform has been established at **https://res4stres.de/** also providing an internal secure environment for data exchange among collaboration partners. High-quality reference genomes were assembled with Oxford Nanopore Technologies (ONT) for 3 selected genotypes.

We expect to identify climate-robust resistances against the targeted insect pest species providing an invaluable resource for future rapeseed improvement particularly under low cropping intensities. Metabolomic pathway analysis will enable us to transfer knowledge also from wild species to *B.* napus to provide metabolome bio marker and molecular marker assays for use in marker-assisted introgression breeding of oilseed rape by the breeding industry.

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Barley Co-Pan-Genome – Computational inference of GxGxE interactions in barley

Aurélien Tellier

A. Tellier[1]

The interaction of crop host genotypes with pathogen genotypes determines resistance of crops and the severity of epidemics by under a given climatic/environmental condition. These Genotype x Genotype x Environmental (GxGxE) interactions determine the resilience of future disease resistance in crops. We focus on barley and two of its main (most complex) pathogens *Fusarium graminearum* responsible for the Fusarium Head Blight (FHB) also characterized by the production of consumer-harmful mycotoxins, and Ramularia *collo-cygni* the agent of Ramularia leaf spot (RLS). Our working hypothesis states that new methods of Genome-to-Genome associations (GtoG or co-GWAS) jointly using barley genetic diversity (SNPs in the barley pan-genome, or Copy Number Variants; CNVs) and pathogen diversity (SNPs and CNVs) across different environments provide a new catalogue of novel resistances, specific or resilient to climatic factors. We build a unique combination of expertise ranging from theoretical evolutionary biology, statistical inference, population (pan)genomics, molecular phytopathology, remote sensing methodology, and plant breeding.

In our barleyCOPA project we first started to derive a novel GtoG framework to analyze the barley and pathogen sequence data. The theoretical work deals with developing a computer simulator of plant-pathogen interactions with diploid host resistance (and dominance). We also develop an epidemiological model of disease transmission and multiple strain infection integrated into an Approximate Bayesian Computation framework to perform GxG association analyses. Second, we have also genotyped (with 15K SNP array) a set of 200 barley lines to be included in our trials. We present the first analysis of relatedness and spatial structure, which guides our further sequencing efforts.

Third, preliminary field trials with 100 genetically diverse barley lines were conducted in 2025 to assess the diversity of pathogen strains to be obtained. Field trials were planted in Kiel, Triesdorf and Freising. We have collected isolates of *Fusarium* and *Ramularia* across fields. We are conducting the (long-read) sequencing of reference genomes for several Fusarium strains of the species complex found on the barley grains. In addition, we have conducted in Triesdorf the first assays of disease severity monitoring by remote sensing methods (drones, hyperspectral cameras) which will be compared to qPCR quantification from infected grains. In 2026, we will conduct the full set of trials with 200 barley lines as well as collection of >1000 pathogen isolates. Using our novel GtoG method we will predict genes underpinning barley resistance to given pathogen strains and its robustness to environmental conditions.

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Priming for enhanced defense as a strategy to optimize crop resistance and as a possible breeding target

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Plant-associated microorganisms play an important role in the ongoing effort to establish more sustainable plant production systems. Beneficial microorganisms have the potential to enhance plant growth and increase plant's resilience. Priming for enhanced resistance, also known as induced resistance (IR), is a promising component of disease management strategies and can be triggered by beneficial microorganisms. In the PrimedPlant project, we focused on bacterial strains applied to barley plants. We performed a long-term field experiment in which several genetically diverse spring barley *(Hordeum vulgare)* lines were inoculated and monitored throughout the vegetative season, with a particular focus on the naturally occurring leaf diseases. We demonstrated that the plant's responsiveness to an inoculation depends on the genetic background of the host. Notably, the naturally occurring infection represents diverse pathogens. We therefore assess how host responsiveness modulates the disease outcomes under dual infection across different barley lines. In addition, we aim to understand the genetic background of the AHL-priming phenomenon. To this end, we performed multiple GWAS analyses combining linear mixed models and machine learning methods to capture complex genotype-phenotype relationships. Together with previously identified QTLs, our results contribute to our understanding of IR mechanisms and support the application of priming in agriculture.

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Epigenetic Editing of Intron Methylation as a Strategy to Modulate Barley Immunity Against Powdery Mildew

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The ongoing loss of biodiversity has intensified social and political pressure to transition toward pesticide-free agricultural systems that safeguard non-target organisms and the environment. To meet this demand, innovative and selective crop protection strategies are required. One promising strategy is CRISPR/Cas9-based epigenetic editing (EpiEdit), which allows precise and reversible regulation of gene expression without altering the DNA sequence. In this project, the molecular mechanisms of transcriptional gene regulation are investigated to establish a sustainable and environmentally friendly platform for crop protection. A modified CRISPR–dCas9 system, in which a nuclease-inactive dCas9 is fused to an epigenetic modifier, was used to achieve targeted (de) methylation of specific genomic loci associated with plant defense. Using this approach, the activation or repression of defence-related genes can be fine-tuned to enhance disease resistance in crops. Barley *(Hordeum vulgare)* infected with powdery mildew *(Blumeria graminis f. sp. hordei,* Bgh*)* was used as a model system. Genome-wide DNA methylation was analyzed in samples collected at multiple time points up to 7 days post-infection and compared with non-infected controls, revealing differentially methylated regions (DMRs). The DNA methylation data were further integrated with RNA-seq and ATAC-seq datasets, providing insights into coordinated transcriptional and chromatin accessibility changes during pathogen challenge. Differentially expressed genes (DEGs) implicated in immune responses were identified by transcriptome analysis and correlated with DMRs to define potential EpiEdit target loci. Although we found only a small fraction of DMRs overlapped with DEGs, this result highlights the complex, multilayered relationship between DNA methylation and gene expression. Notably, intragenic CG methylation, particularly in introns, was associated with attenuated gene induction upon infection. This observation suggests that intron methylation functions as a quantitative modulator of transcriptional responsiveness, offering a potential mechanism for the epigenetic fine-tuning of defence gene activity. Based on these findings, pathogen-responsive genes displaying intron methylation and moderate transcriptional changes were selected as candidates for EpiEdit loci and targeting. Overall, our study advances the mechanistic understanding of the interplay between DNA methylation and gene expression in plants, establishing a conceptual and technological foundation for the precise epigenetic control of crop immunity. These findings may ultimately contribute to the development of next-generation crop protection strategies that enhance disease resistance through programmable, heritable, yet reversible gene regulation, promoting more resilient agricultural systems.

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INNO-TOM – Pangenomes and gene targeting to create disease resistant and biofortified cis and intra-genic INNOvative TOMato varieties

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INNO-TOM aims at creating novel tomato cis- and intragenic varieties with traits that will provide benefits for the growers as well as for the consumers. The traits we will target are bacterial and virus resistance as well as biofortification. We will introduce these traits using a highly efficient genome editing technology (Cas-Exo) developed during a previously funded BMBF project (*Genereplace*, Crop Plants of the Future) (Schreiber et al., 2024). Cas-Exo is based on homology directed repair (HDR) and allows the direct replacement of alleles or variants from the breeder's pool into the genome of breeding cultivars. The advantages of the Cas-Exo technology are speed and precision, which avoids the problem of linkage drag in traditional breeding. We target two major diseases of tomato cultivation: bacterial spot (caused by Xanthomonas sp.) and viral diseases caused by Tobamoviruses, such as the Tomato Mosaic Virus (ToMV) and Tomato Brown Rugose Fruit Virus (ToBRFV), which can cause major yield loss. The resistance to ToMV conferred by the *Tm2²* has been overcome by new virus isolates and by the emerging and rapidly spreading ToBRFV. We have tested variants of *Tm2²* affecting the LRR domain in *N. benthamiana* and some of them lead to a hypersensitive response when co-expressed with the movement protein of ToBRFV. These variants are being introduced directly in the tomato genome by gene replacement using Cas-Exo. We employ several complementary strategies to enhance resistance against Xanthomonas in tomato. First, we are establishing luciferase-expressing Xanthomonas strains to enable quantitative screening of tomato accessions for reduced pathogen growth. In parallel, we have developed a transient expression system for tomato that allows expression of individual Xanthomonas effectors in diverse accessions to identify effector-induced resistance responses. To support these screens, Xanthomonas effector gene libraries are currently being assembled in Agrobacterium-compatible T-DNA vectors.

In addition, we leverage our expertise on Xanthomonas TAL effectors to engineer targeted resistance. Specifically, we exploit the widespread TAL effector AvrHah1 by inserting its corresponding target motifs upstream of host cell death–inducing genes, thereby generating TAL-effector "trap" constructs. Furthermore, we apply gene editing to modify the general transcription factor TFIIAγ, a docking factor that TAL effectors interact with to manipulate host transcription. We have successfully produced tomato lines carrying edited TFIIAγ alleles and confirmed that TAL effectors are unable to manipulate host cells in these plants.We also aim to create cis-genic tomato lines with purple fruits enriched in anthocyanins that provide health benefits, prolong the shelf life of tomato fruits and reduce the susceptibility to grey mold. For this, transcription factors of the MYB (e.g. AN2) and bHLH (AN1) type have to be overexpressed in the fruit. We are targeting the promoters of these genes by integrating fruit specific promoters such as the E8 promoter. Finally, we will assemble a tomato pan-genome including many publicly available tomato accessions as well as tomato varieties of direct interest for the project. This pangenome can be explored on a user-friendly browser named Pantograph. This will allow the consortium partners to easily interrogate tomato genomes for sequence and structural variants in genes or genomic regions of interest.

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RecREdit – Generation of recessive resistance against aphid-transmitted viruses in sugar beet using new breeding technologies – a case study for the establishment of precise genome modification in a multiuse crop

B. Garz[1], L. Rollwage[1], M. Varrelmann[1]

Since the EU-wide ban on neonicotinoids in 2019, sugar beet production has been increasingly challenged by aphid-transmitted "virus yellows" (VY). Traditionally, the VY complex in sugar beet is dominated by beet yellows virus (BYV), frequently reported as a major contributor, together with the poleroviruses beet mild yellowing virus (BMYV) and beet chlorosis virus (BChV). Additional aphid-borne viruses such as beet western yellows virus (BWYV) and beet leaf yellowing virus (BLYV) are monitored as potential contributors depending on region. The potyvirus beet mosaic virus (BtMV) is aphid-transmitted and therefore closely associated with VY outbreaks, although it typically does not cause the classic yellowing symptoms. Outbreaks of VY can result in substantial yield losses, since aphid pressure and virus levels are difficult to predict and resistant varieties are still limited.

In previous work, we achieved recessive resistance against BChV by knocking out the susceptibility factor eukaryotic translation initiation factor (iso) 4E (Bv-eIF(iso)4E) *(Rollwage et al., 2024)*. However, for BMYV and BtMV no resistance was observed, likely because both viruses can interact with both eIF4E isoforms. Because a double knockout of Bv-eIF4E and Bv-eIF(iso)4E is expected to be lethal, RecREdit focuses on a more sophisticated approach: Identifying amino-acid changes in both isoforms that disrupt binding to the viral protein genome-linked (VPg), a translation-related viral protein that was shown to interact with host initiation factors, while maintaining the normal m7G cap-binding function and plant fitness. This strategy assumes that VPg binding and cap binding depend on partially distinct animo acid determinants within eIF4E/eIF(iso)4E, so we can block VPg recruitment without impairing cap binding.

To increase the precision of previous eIF-poleroviral VPg interaction assays, the exact BChV/BMYV VPg boundaries are determined using transient cleavage assays in planta *(Nicotiana benthamiana)*, which are planned to be supported with protein chemistry approaches e.g. LC-MS/Edman. In parallel, we screen natural eIF alleles and systematically created eIF mutants for loss of poleroviral VPg interaction using established protein-protein interaction assays, including yeast two-hybrid (Y2H) and bimolecular fluorescence complementation (BiFC), and we assess cap binding using a yeast-based cap complementation readout. Structural modelling has been integrated to highlight likely interface residues and to guide which substitutions are promising. Validated substitutions will later be introduced into sugar beet via genome editing.

T2 seeds of eIF-knockout sugar beet were produced. Initial greenhouse workflows have been established, to focus on fitness cost assessments with standardized phenotyping, especially investigating Bv-eIF(iso)4EKO. Furthermore, first virus accumulation experiments are planned to use T2 knockout plants grown from seed, starting with BWYV as a proactive assessment, as BWYV has not been widely reported in the EU to date. First *Bv-eIF*(iso)4E amino-acid substitutions are being tested in yeast-based functional cap readouts. Work is ongoing to refine VPg boundaries and improve assay precision by testing reporter constructs that indicate VPg cleavage. Structural modelling of Bv-eIF(iso)4E with a cap analogue and a putative VPg peptide has generated a first list of promising candidate residues predicted to contribute to VPg binding while being less critical for cap binding.

Overall, RecREdit aims to provide sugar beet lines with recessive resistance against multiple aphid-transmitted viruses and minimal fitness penalties by combining VPg identification, interaction screening, and precise genome editing.

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KEYNOTE: Designing and delivering future sustainable wheat: a coordinated UK research programme

Malcolm J Hawkesford[1]

In the UK, the Biotechnology and Biological Sciences Research Council (BBSRC) funds strategic research on wheat improvement, most recently through the Designing Future Wheat (2017-23) and Delivering Sustainable Wheat (2023-2028) programmes. These are coordinated projects involving multiple research institute and university partners. A key aspect are the pathways to impact, via interaction with industry including commercial breeding companies.

Initially a major activity was the assembly of wheat germplasm resources encompassing the widest genetic diversity possible, including modern elite varieties, historic varieties, landraces, wild relatives and new synthetic hexaploids. Genetic resources were further developed from this material by crossing and introgression into adapted modern varieties. One example is the Watkins landrace collection, which has been examined by sequencing and phenotyping, and for which multiple biparental mapping and NAM populations have been created (Cheng et al 2024).

Key traits of interest comprise sustainability, resilience and nutritional traits. Examples include genotypic diversity in root soil interactions investigated in trials with differential tillage, combining soil science and plant biology approaches (Raza et al 2025). Variation in and climate resilience of canopy and reproductive organ development are modelled and dissected at the molecular level. Given the nutritional importance of wheat, mineral content, soluble fibre and starch content are all key targets for improvement. In the case of soluble fibre (Shewry ey al 2024), genetic and biochemical analysis of wheat flour will be complemented by clinical studies of the positive impact of increased fibre content.

The extensive field trialling has required the development and deployment of new high-throughput phenotyping tools for root, canopy and grain screening including dron-based and automated robotic systems (Virlet et al 2017), and bespoke computer vision processing pipelines.

Underpinning the programme is a commitment to FAIR data and an example is the GrassRoots database system (Grassroots) in which experimental result are deposited.

Partners

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EpicBeet – Stability and heritability of DNA methylation patterns in sugar beet – impact on phenotypic plasticity, influence on TE activity and potential for beet breeding

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Although epigenetic modifications are causal to some plant phenotypes, epigenetically informed selection is not yet integrated in modern breeding. Potentially, DNA methylation accounts for the lack of heritability observed after genomic selection. However, only regions with stably inherited DNA methylation are useful for epigenomic selection. As the DNA methylation's stability and heritability have been investigated only scarcely, and solely for model plants, the fundamental basis for epigenetic crop breeding is still lacking. On top, as perturbance of DNA methylation is often followed by transposable element (TE) mobilization, it will also yield genetic variation.

In the EpicBeet project, we focus on the biannual crop sugar beet *(Beta vulgaris ssp. vulgaris)*. Its genome is diploid and therefore comparably small, has an annotated reference genome sequence [1], and has highly methylated [2], well-characterized repeats [3, 4]. To study epigenetic heritability, we focus on sugar beet mutants with perturbed DNA methylation. Overall, we aim to provide clear recommendations for the setup of epigenetic breeding lines as well as to generate fundamental, new information to better understand the heritability and stability of DNA methylation and its impact on the TE landscape in the sugar beet crop.

In the first project phase, we have screened more than 12,000 EMS-mutagenized plants to identify mutants in the hypomethylation-associated candidate genes *Decrease in DNA methylation (DDM1), Methyltransferase 1 (MET1)* as well as in the hypermethylation-associated gene *MutS Homolog 1 (MSH1)*. This screening resulted in a pool of seven different STOP and 61 different point mutations. For some of them (one STOP and fifteen point mutations) it was possible to generate homozygous mutant as well as epihybrid states.

Apart from generating material, we now have characterized the many mutants as well as the corresponding parental plants on a phenotypic, molecular and (epi-)sequence level using Oxford Nanopore Technologies (ONT) and enzymatic methyl-sequencing. To date, Illumina short read and methyl-sequencing data have been generated for 34 (epi)genotypes, whereas 26 were ONT-sequenced. To streamline the computational analyses, we have advanced the technology involved in generating ONT-based assemblies of beets as well as using them to infer DNA methylation and presenting the sugar beet reference methylome [2]. We have also curated our beet repetitive DNA reference libraries [4] and screened the sequenced sugar beet lines for TE polymorphisms. To better evaluate associated phenotypes, we are currently phenotyping 150 EpicBeet plants in the DPPN phenotyping facilities.

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Characterizing transcriptomic drivers of microspore reprogramming

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Doubled-haploid technology provides enhanced access to genetic variation while simultaneously generating lines that are genetically fixed at every locus within a single generation. For doubled-haploid plant generation, microspores (cells of the male gametophytic lineage) have to reprogram their developmental fate from pollen to embryo formation. Recent evidence suggests that perturbations in epigenetic pathways can serve as powerful tool to enhance this gametophytic reprogramming.

Aim:
EpiHAP proposed following the differential fate of single cells/nuclei during the process of gametophytic reprogramming to better understand the response to inducing cues. The long-term goal is to use this knowledge to design treatments that increase the efficiency of gametophytic reprogramming.

Approach:
We employed a single-nuclei RNA sequencing (sn-RNAseq) approach to characterize transcriptomic changes occurring at the level of individual cells during reprogramming. Microspores of Brassica napus were subjected to a 3-day heat shock treatment, and transcriptomes of single nuclei were generated at different time points (day 0, day 1, and day 4).

Results:
Integrating of all datasets allowed us to identify distinct subpopulations within the nuclei. Based on previous knowledge, we could clearly associate several clusters to generative and vegetative cells, as well as uninucleate microspores at various stages associated with cell cycle progression. A cluster of responsive cells was detected from day 1 after induction and expressed embryogenesis-associated genes, in particular at day 4. The predominant gene ontology terms associated with the responsive cell cluster at day 1 and 4 related to ion homeostasis and transport. Using pseudo-time analyses, we are currently trying to understand from which pool the number of responsive cells is recruited; the most probably candidates being pre-mitotic micro spores. Importantly, the cluster of responsive cells was distinct from a population most responsive to the inductive heat stress, indicating that the response is not proportional to the severity of the stress.

Conclusion:
A population of treatment-responsive cells was identified after 1 day, thus prior to observable visible changes indicative of gametophytic reprogramming. Instead of the induction of stress genes, the cells appear to actively establish or maintain ion homeostasis, indicating that chemical stimulation of ion transport could be explored for improved gametogenesis efficiency in the future.

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EpiSOMA – Environmentally induced somatic epimutations in trees and their potential link to seedling drought resistance

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Background
Trees accumulate epimutations throughout their lifetime, and experimental evidence suggests that epimutations may function as a short-term adaptive mechanism in stressed plants. Recent large-scale climate-induced forest diebacks indicate that climate trends are imposing increasingly frequent and severe stress on forest ecosystems. Understanding the consequences of environmentally induced epimutations could therefore help clarify the potential role of epigenetic mechanisms in the response of long-lived forest trees to climate change.

Aims
Within the epiSOMA project, we used European beech (Fagus sylvatica) as a model species to test two hypotheses. First, we hypothesized that individual trees exhibit differential patterns of somatic epimutations between sun-exposed and shaded crown positions given the differential microclimates associated with the tree vertical gradient. Second, we hypothesized that some of these differential patterns can be transmitted to the next generation in tree populations growing in contrasting environments (dry vs. moist forest plots), potentially conferring greater drought resistance to seedlings originating from dry plots.

Methods
We studied environmentally induced somatic epimutations with a within-tree approach and assessed their heritability with an experimental approach.The within-tree approach included quantifying microclimatic differences, monitoring spring phenology, and contrasting leaf DNA methylation (via Whole Genome Bisulfite Sequencing – WGBS) between sun-exposed and shaded crown positions.For the experimental approach, we collected freshly germinated seedlings from contrasting environments in Kellerwald-Edersee National Park: dry, south-facing slopes with shallow soils and moist forest plots with closed canopies and used them in a drought stress experiment. This experiment consisted of controlled drought and light treatments and was implemented at the EcoSystem Analyser (TUMmesa) during nine weeks.

Findings
We detected differentially methylated regions (DMRs) between leaves from sun-exposed and shaded crown positions within each tree, , predominantly reflecting methylation gains in the CpG context. Despite the common pattern of higher global methylation in the CpG sequence context in leaves of the shaded crown positions, no DMR was shared across all three trees.Seedling origin also influenced water-use efficiency under drought conditions. The isotopic composition of leaves developed during the experiment revealed that seedlings from moist plots exhibited pronounced plasticity in heavy carbon enrichment ($\delta^{13}C$) under stress, whereas seedlings from dry plots maintained consistently high $\delta^{13}C$ values. Consequently, seedlings originating from dry forest plots displayed a drought-resistant isotopic profile relative to those from moist plots, particularly under high-light conditions simulating dry-plot environments.Integrative omics analyses are currently underway to link seedling phenotypic plasticity with underlying epigenetic variation.

Conclusions.
Somatic methylation patterns differ between crown positions within trees, but these differences are largely tree-specific and do not reveal a uniform epigenetic signature associated with crown microclimate differences. Seedling origin from contrasting environments influences water-use efficiency under drought, indicating possible environmental priming independent of detectable genetic differentiation.

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SEEDMAKER – Deciphering the epigenetic mechanisms necessary to engineer apomictic endosperm development in crops

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Clonal seed production, also called apomixis, has long been viewed as the Holy Grail of plant breeding. This is because apomixis would allow the fixation of particularly high agronomic performance based upon hybrid vigour, and thus bypass costly hybrid seed production. Additionally, clonal seed development would allow to cope with adverse environmental factors, like drought or absence of pollinators, that limit plant fertility and therefore yield. Nevertheless, apomixis is not present in any major agricultural crops, and its introduction via traditional breeding methods has proved unsuccessful. Full apomixis necessitates three independent pathways: bypassing meiosis, parthenogenic embryo development and autonomous endosperm development. And while recent developments have allowed to produce clonal parthenogenic embryos at high frequency, the development of an autonomous endosperm remains an outstanding challenge. Our project thus aimed at identifying genetic determinants of autonomous endosperm formation, which are under the control of two epigenetic marks: the trimethylation of lysine 27 on Histone 3 (H3K27me3), and DNA methylation (mC). For this, we used the model plant species Arabidopsis thaliana. In parallel, we produced tools to engineer autonomous endosperm in barley. Namely, we generated transcriptomes of various ovule and pericarp tissues from dissected barley grains during development, which we used for gene network analyses and to identify and annotate barley DEGs and homologues of the most promising candidate genes identified in Arabidopsis. These approaches allowed unfolding the dynamic interplay of maternal-filial genes with key functions during grain development, as well as identifying ovule-specific promoters that can be used to drive the expression of determinants of apomixis. With this, we aim to generate tools that can be used to engineer autonomous endosperm in a cereal, thus eliminating the last barrier for the introduction of full apomixis into an important crop.

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Potatoes for tomorrow – Improving genetic traits using potato genetic resources and new breeding techniques

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The POMORROW project aims to enhance the sustainability and resilience of cultivated potato by developing all of the components required for an efficient exploitation of potato genetic resources (PGR) ensuring future breeding gain. While safe-guarding PGR has a merit on its own, it is only by use in crop improvement programs that PGR unfurl their full potential as reservoir of genetic variation required for addressing future challenges in crop production. The GLKS potato collections of the German Federal Ex situ Gene Bank at Leibniz IPK will be completely genotyped. A POMORROW core collection (PCC) of 600 entries will be established and extensively phenotyped for emerging and underexplored traits that are of particular relevance for potato production under future environmental and regulatory constraints. Traits include e.g. drought tolerance and its interaction with response to arbuscular mycorrhiza (AM), nutrient use efficiency, nutritional value and resistance to late blight, stolbur/*Arsenophonus*, and potato viruses. Association genetics in PCC, as well as novel diploid community germplasm resources, will enable identification and validation of valuable alleles for potato breeding. In order to facilitate the use of genetic resources for breeding, new biotechnological tools for transgene-free gene editing are investigated and developed. Finally, to further improve potato cultivars with PGR, the potato breeders' toolbox will be complemented by predictive breeding approaches to enable for the first time the exploitation of minor effect loci. Taken together, POMORROW by harnessing genetic resources develops and applies conventional and novel breeding techniques for potato germplasm enhancement, which allow potato breeding to react rapidly to new challenges posed by climate change and the bioeconomy.

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DPPN-ACCESS – Providing access to state-of-the-art phenotyping infrastructures

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The German Plant Phenoytyping Network (DPPN) has developed and established state-of-the-art infrastructures with a number of installations addressing questions that require quantitative plant phenotyping of different crops under various environmental scenarios **https://dppn.plant-phenotyping-network.de**. The DPPN partners, Forschungszentrum Jülich, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben and the Helmholtz Munich founded the DPPN e.V. to provide access to users from German academia (mainly universities) to the plant phenotyping installations, techniques and methods of the
partners of the association.

The DPPN e.V. acquired the BMFTR-funded pilot project “DPPN-ACCESS” (01.01.2022 – 31.12.2023) where the requirements for the application procedure and the application guidelines were drawn up and first accesses were successfully provided. Further external user projects in the longer term are ensured by the BMFTR-funded follow-up project “ DPPN-ACCESS 2.0” (01.01.2024 – 31.12.2028). The talk will give an overview of the current project status of “DPPN- ACCESS”, outline the available facilities, summarize the access projects that were enabled so far and, sketch how to further benefit from access to the facilities. The application procedure will be described in detail. A new call for applications is currently open, with a deadline for submission until April 14 **https://dppn.plant-phenotyping-network.de/Access_Calls**. Further calls will be launched approximately every six months.

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SorBOOM – Sorghum – boosting breeding by multilevel modelling

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SorBOOM aims at boosting grain sorghum breeding for Central European target environments via modern breeding technologies, multilevel modelling and use of big data.

Field trials comprising 195 experimental F1 hybrids, 45 parental lines and several commercial checks were successfully conducted in 2025 at four locations (2 French and 2 German). Trials at the German locations (Gross-Gerau and Quedlinburg) were scored once per week with multispectral sensors (UAV) to determine vegetation indices (NDVI, NDRE, GNDVI). Previously developed software pipelines will be applied on the images to create orthomosaics and extract genotype-specific reflectance data which can be used to calculate vegetation indices. The yield data of all 4 locations show a high genotype x environment interaction as expected. 24 experimental hybrids outyielded the best performing check *Huggo* regarding their mean over locations. In 2026, the better performing ~50% of these experimental hybrids will be re-tested, including two locations in Italy, while the lower performing 50 % will be replaced by new hybrids which are currently being produced in a winter nurserey in Mexico.

The first drought stress experiment comprising 20 genotypes (7 restorer lines, 2 female lines, 10 experimental F1 hybrids and one commercial check, 3 replications per treatment) was conducted in the PlantArray facility in Quedlinburg in 2025. This system allows precise monitoring of the daily water loss per pot through transpiration. Further, to establish a detailed scoring protocol of sorghum root morphology, two preliminary experiments with nine parental sorghum lines and one commercial variety were conducted in a hydroponic system which had been efficiently utilized for other cereals previously. In 2026, a large hydroponic experiment including ~250 genotypes (those tested in the aforementioned field trials) is carried out utilizing the now established protocol.

Regarding multilevel modelling, the JKI Institute for Strategies and Technology Assessment conducted a detailed parametrization field trial in Dahlem to generate data for training a process-based model called DSSAT *(Decision Support System for Agrotechnology Transfer)*.

The scientific position has just been filled recently (January 2026) to work on the generated data. As the project is still in an early phase, data analysis and generation of first results are ongoing.

In the agrobioinformatics part, haploid-resolved genome assemblies were generated using PacBio HiFi data with hifiasm. These assemblies were error-corrected and scaffolded using the in-house developed *noHiC* pipeline. For genome annotation, we benchmarked *Tiberius* (trained on sorghum) against BRAKER3, which was trained using publicly available sorghum RNA-seq and protein datasets. BRAKER3 showed higher completeness and was therefore selected for downstream annotation. For 66 assemblies, the annotation is currently ongoing. Structural variant (SV) calling is being performed using assembly-based approaches. Further, RNA samples were collected from all genotypes at the Gross-Gerau field trial during booting stage. RNA isolation and sequencing are planned to be completed in the second quarter of 2026.

Regarding genome editing for zero-tannin, we optimised the protocol for sorghum tissue culture by testing different explants (immature embryos, leaf tissue and hypocotyls) and media for efficient callus induction and plant regeneration. Further, we tested the production of Cas9-expressing plants via particle bombardment and are running the respective screening right now. Positive plants will be used for CRISPR/Cas9 editing via viral vectors. Another working package which is currently being carried out is the CRISPR-component delivery via RNPs, which would allow for a transgene-free gene editing.

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Twin – A Digital Discovery Platform for Cereal Genetic Resources

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Background and Motivation
Crop genetic resources conserved in genebanks represent a unique repository of evolutionary and adaptive diversity, yet their systematic utilisation in research and breeding remains limited by fragmented genomic and phenotypic information. Recent advances in whole-genome sequencing, pangenomics, and data science now enable a transition from static collections to integrative, data-driven discovery systems.

Concept and Objectives
The Twin project establishes the conceptual and technical foundation for *digital twins* of cereal genetic resources. Focusing on barley *(Hordeum vulgare)* and wheat *(Triticum aestivum)*, Twin creates dynamic digital counterparts of genebank accessions that integrate genome sequences, pangenome context, phenotypes, and environmental metadata. These digital twins form a continuously evolving discovery platform that links conservation, functional genomics, and translational breeding.

Data Generation and Infrastructure
Twin will generate whole-genome sequence data for approximately 15,000 non-redundant barley accessions (~10× coverage) and 1,500 representative wheat accessions. The resulting variant, haplotype, and structural-variation datasets are integrated into existing barley and wheat pangenomes. FAIR-compliant data architectures, databases, browsers, and APIs provide sustainable access and interoperability with national and international infrastructures.

Data Science and AI-Based Analysis
Advanced bioinformatics and artificial-intelligence approaches form the analytical backbone of Twin. Deep-learning models and genomic language models are applied to genome annotation, regulatory-element inference, and variant-effect prediction, enabling improved functional interpretation at genebank scale. The resulting pan-regulome resources link sequence variation to gene regulation, expression, and phenotype.

Translational Case Studies
The utility of the digital-twin framework is demonstrated through targeted biological case studies. In barley, Twin investigates the genetic and metabolic basis of hordatine-mediated defence and natural variation in meiotic recombination landscapes. In wheat, integrative population-genomic and transcriptomic analyses identify adaptation, resistance, and reproductive-development genes relevant for breeding.

Impact and Outlook
Twin establishes a scalable, interoperable blueprint for digital genebank resources, transforming preserved diversity into an active, predictive research infrastructure. By coupling large-scale genomics with AI-driven analysis, the project advances Germany's leadership in plant genetic-resource digitalisation and provides a foundation for next-generation, data-informed breeding strategies.

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Poster presentations

PREBreed – Leveraging functional pan-genomics for enhanced breeding research in barley

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Generating accurate and comparable gene predictions across diverse genotypes is crucial for utilizing pan-genomes in crop improvement. PREBreed will integrate high-resolution de novo annotation, co-expression network analysis and high-throughput functional validation to translate the genetic diversity of barley into breeding-ready traits. Current pan-genome annotations often rely on gene projections which may overlook genotype-specific genes, such as those from wild forms. To fix this bias, PREBreed will expand the current barley pan-transcriptome to generate evidence-based transcriptomes for 56 additional barley genotypes using RNAseq and ISOseq. This will encompass the embryo, shoot, root, inflorescence, and caryopsis to generate an orthologous framework for the 76 genotypes of the Barley Pangenome V2. By constructing genotype-specific co-expression networks, we will identify core gene modules controlling vital processes like photosynthesis, carbohydrate metabolism, and nutrient uptake. This resource will allow investigation of cis-regulatory elements and the selection of candidate genes for subsequent functional analyses. PREBreed translates bioinformatic predictions into quantifiable phenotypes using the non-GM, high-throughput FIND-IT (Fast Identification of Nucleotide variants by droplet DigITal PCR) technology. Screening elite mutant libraries of over 500,000 individuals offers a powerful alternative to traditional TILLING and CRISPR-Cas9 mediated mutagenesis. The project will isolate 400 mutants to characterize allele function and potentially induce gain-of-function variants. Through these activities, we will target traits associated with nitrogen use efficiency, root development, and carbon assimilation, ensuring that the diversity captured in the barley pan-transcriptome is accessible for precision breeding and climate-resilient crop development.

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PREBreed – A Web Portal for Project Results with Integrated Material and Sample Registration

Stefanie Lück[1], Danuta Schüler[1], Uwe Scholz[1]

Within the PREBreed project, Partner II (IPK–BIT) contributes a digital infrastructure to support coordinated data and material management. A central web portal provides project-wide information, including work package descriptions, partner details, and project structure, and will be progressively extended to integrate datasets generated during the project, ensuring transparent access and long-term usability (WP4), in line with the FAIR data principles (Findable, Accessible, Interoperable, Reusable). Within WP3, a project-wide material and sample registration framework is established. Preserved accessions of plant genetic resources serve as the primary biological materials and originate from genebank collections. Material metadata include organism characteristics, genebank accession identifiers, cultivar and taxonomic information, geolocation and source material collection data, donor institute identifiers, and additional persistent identifiers (e.g. DOI), with accession-level metadata sourced from external systems such as GBIS/I and EURISCO. Each registered material is assigned a unique PREBreed material identifier and can give rise to multiple biological samples, resulting in a one-to-many (1:n) relationship. Samples represent experiment-specific instances of a material and receive unique PREBreed sample identifiers linked to their parent material. Sample metadata obtained from individual plants include organism characteristics, sample collection data (age, developmental stage, and plant part), cultivation information (growth medium, growth facility, pH, temperature), local environmental conditions, and associated LIMS sequencing identifiers. Samples derived from leaf, seed, or stem are used for downstream analyses including phenotyping, microscopy, sequencing, and microdissection. Data management in PREBreed is restricted to datasets associated with uniquely registered materials and samples. Sample-level data, including phenotypic, genotypic, molecular, and imaging datasets, are managed via project-specific infrastructures and interoperable repositories such as the IPK system LIMSOPHY and the EMBL repository BioSamples. This structured approach ensures traceability, consistency, and reproducibility across partners and provides the basis for sustainable data integration, visualization, and long-term reuse.

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Phenotypic Plasticity and Genetics of Drought Stress Adaptation in Barley Roots

Prof. Dr. H. Schneider[1], P. Hampe[1]

Barley *(Hordeum vulgare L.)* is one of the earliest domesticated crops and remains one of the most important cereals cultivated worldwide (Lukinac & Jukić, 2022). While primarily used for feed and malt, it holds significant potential for food production (Sharma & Gujral, 2010) and serves as a valuable model for climate change adaptation (Dawson et al., 2015). Compared to above-ground traits, root systems have historically been understudied due to the challenges of soil accessibility (Jia et al., 2019). However, recent evidence highlights the critical role of root phenotypic plasticity (Schneider & Lynch, 2020) and specific root traits in crop breeding (Lynch et al., 2021).

This study explores the genetics underlying barley root phenotypic plasticity in response to drought stress through three primary objectives. First, we aim to characterize the genetic control of root anatomy and drought response using the barley pangenome panel (Jayakodi et al., 2024), which encompasses diverse genotypes and elite germplasm. To address the first objective, preliminary experiments have been initiated to characterize root anatomy and architecture in solution culture. Second, we will identify quantitative trait loci (QTL) governing root traits under progressive water stress using 200 recombinant inbred lines (RILs) from a Morex x Barke (F11) population. Third, experiments are planned to validate candidate genes for drought tolerance using 400 NaN3- induced mutant lines from the FIND-IT™ platform. Ultimately, this research aims to develop a breeding toolkit by cataloging allelic diversity associated with adaptive stress tolerance.

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Uncoupling yield-enhancing dwarfism from low nitrogen efficiency in barley

L. Yang[1], S. Jhingan[1], J. Szymanski[1], N. Stein[1], C. Dockter[2], N. von Wirén[1]

During the Green Revolution, the introduction of SEMI-DWARF genes, such as wheat *Reduced height1 (Rht1)* or rice *semidwarf1 (sd1)*, significantly enhanced grain yield by improving harvest index and lodging resistance, conferred by reduced plant height. Unfortunately, studies in rice have shown that these dwarfing genes have a negative effect on plant nitrogen uptake capacity, resulting in a higher demand for nitrogen fertilization. Here, we investigate the genetic basis underlying plant height regulation and nitrogen uptake in barley *(Hordeum vulgare)*, aiming to uncouple yield-enhancing dwarfism from low nitrogen use efficiency (NUE) in barley cultivars. Through detecting nitrogen-related traits in a panel of 209 European two-row spring barley cultivars, which represent the pan-European breeding progress from 1830 to 2014, we found that modern cultivars tend to have lower nitrogen uptake capacity, while no such trend was observed for shoot nitrogen content. We then conducted genome-wide association studies (GWAS) and identified a shared quantitative trait locus (QTL) associated with nitrogen uptake capacity and shoot nitrogen content. The strongest association signal was located in the terminal region of chromosome 2H. Within this interval, we identified *GA3-oxidase 1 (HvGA3ox1)*, a key enzyme in gibberellin biosynthesis, as promising candidate gene that also exhibits nitrogen-responsive expression in roots. Haplotype analysis further revealed an elite allele of *HvGA3ox1* associated with enhanced nitrogen uptake capacity without detectable effects on plant height compared to other haplotypes. Additionally, ammonium uptake capacity is reduced in these mutants, with ongoing experiments investigating their nitrate uptake capacity. The integration of additional gibberellin-related mutants will further clarify the mechanistic links between gibberellin metabolism, root development, and nitrogen uptake.

Keywords
Barley, gibberellin, nitrogen use efficiency (NUE), plant height, nitrogen uptake

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Exploring alternative ways to transform barley

A. Ziplys[1], J. Schippers[2]

The successful pangenome projects in barley have uncovered a tremendous genetic diversity, which requires a detailed functional analysis of candidate genes by using transgenic approaches through efficient and cost-effective transformation pipelines. Barley transformation relies on culture systems that regenerate plants from transformed calli or cells. Although this procedure is well established in barley, it is mainly limited to the cultivar (cv) Golden Promise, while many other cultivars remain recalcitrant to this method. Here we aim at establishing novel barley transformation protocols by systematically testing alternative approaches1. Novel, easier and broader applicable methods would open up possibilities for large-scale functional genomics studies.

A game changer for barley would be a method that is as effective as the floral dip transformation of Arabidopsis, which enabled high-throughput functional genomics resulting in most of our current molecular and genetic understanding of plants. Here we test the option for establishing Hordeum vulgare Injection Transformation (HIT) as as an alternative method for generating transformants. In principle we inject various barley cultivars at different stages with Agrobacterium strains containing a reporter plasmid. Subsequently, progeny of these plants will be screened for transformation events.

Here a short overview of the current strategy and preliminary results will be presented.

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Predicting Expression and Regulatory Motifs in Barley with the DeepCRE toolkit

Simon Maria Zumkeller[1], Tobias Pfeifer[2], Jedrzej Jakub Szymanski[1,2]

Understanding the relationship between non-coding regulatory sequences and gene expression is fundamental to decoding plant regulation and genetic variation. We previously established a framework using interpretable deep learning models to predict expression profiles from flanking DNA regions with over 80% accuracy. Here, we present an expanded, interactive web-based toolkit that significantly broadens its utility for the cereal research community by integrating high-performance models for sweet-grasses, including *Zea mays* (maize), *Sorghum bicolor* (millet), *Oryza sativa* (rice), and *Hordeum vulgare (barley)*.

A key advancement in this version is the ability to directly investigate barley genes of interest using the Morex reference genome. The toolkit now features an integrated deepCRE annotation tool, which allows users to identify and download expression predictive motifs—such as transcription factor binding sites and salient cis-regulatory elements—directly within the browser. By combining these predictive features with existing functions like in silico mutational analysis and VCF-based variant effect prediction, this platform provides an accessible, end-to-end entry point for Barley researchers to decode the regulatory logic driving phenotypic and metabolic traits in major cereal crops.

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PREBreed – Annotating the next generation of the barley pan-genome

Thomas Lux[1], Georg Haberer[1], Srijan Jhingan[2], PREBreed Consortium, Uwe Scholz[2], Martin Mascher[2], Nils Stein[2], Klaus F.X. Mayer[1], Manuel Spannagl[1]

To assess the gene content in the BPGv2 barley pan-genome we employed a multi-tier gene prediction strategy (Ref_1). In a first step, comprehensive de-novo gene predictions were carried out for 20 barley genotypes with extensive native transcriptome data. In a second step, the non-redundant union of these gene predictions was projected to the genome assemblies of 56 barley genotypes without native transcriptome data, using a newly developed gene projection procedure. This procedure provides a robust basis for comparable, downstream genomic analyses and the definition of the barley pangenome.

PREBreed will expand the current barley pan-transcriptome to generate evidence-based gene predictions for 56 additional barley genotypes using RNAseq and ISOseq. This dataset includes embryo, shoot, root, inflorescence, and caryopsis. Based on the individual lineage gene sets we will construct a high-density orthologous framework which defines the gene-based next-generation barley pangenome.

In addition, we here present an evaluation of the gene projection approach, as well as a comparative assessment of the impact of long-read transcriptome data on gene prediction accuracy.

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Guide RNA – The molecular GPS of the Cas endonuclease complex

S K. Nimmalagottu[1], R. Hoffie[1]

CRISPR–Cas-based genome editing technology, originally derived from a bacterial adaptive immune system, has become a powerful tool in plant biotechnology, enabling precise and site-specific genetic modifications for crop improvement. The Cas complex relies on a customizable guide RNA (gRNA) to direct the Cas nuclease to specific genomic loci defined by the presence of a protospacer adjacent motif (PAM). However, inefficient gRNA design can significantly reduce editing efficiency. The secondary structure of the gRNA is critical for Cas binding and activity, as conserved stem–loop structures mediate stable Cas–gRNA interactions. Intramolecular interactions between the target-specific guide sequence and scaffold region can disrupt proper folding, reducing nuclease affinity and function. Therefore, optimizing gRNA structure is essential to enhance editing performance. This study examines the impact of gRNA scaffold optimization on editing efficiency and target accessibility. Disruption of cryptic stop codon through targeted base exchange at the poly 'T' stretch minimizes premature transcription termination. Additionally, stem–loop extensions enhance scaffold stability by maintaining proper secondary structure, which facilitates access to genomic regions that are otherwise intractable. Considering these manipulations to the scaffold, five gRNA scaffold variants were selected and successfully cloned to introduce individual changes relative to the wild-type scaffold. A complementary strategy involving deliberate single-nucleotide substitutions allows the development of customized gRNAs tailored for a particular target motif. Overall, these optimization strategies are believed to enhance the precision and efficiency of CRISPR-mediated genome editing, particularly benefiting advanced applications such as base and prime editing.

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Precise genome editing with Cas endonucleases – focus on the role of a fusion partner

H. Hecht[1], J. M. Spieß[1], R. E. Hoffie[1]

Achieving precise and efficient genome editing remains a challenging goal in plant biotechnology. Established methods, such as CRISPR-associated (Cas) endonucleases, base editing, prime editing and homology-directed repair (HDR), are limited in terms of either accuracy or efficiency. The predominant DNA repair pathway is non-homologous end joining (NHEJ) that usually leads to small insertions or deletions, when erors occur upon repair, leading to frameshift mutations that knock out the gene's function. For precise editing, microhomology-mediated end joining (MMEJ) is a promising alternative., That endogenous repair mechanism acts independently of the cell cycle and uses microhomologies near the double-strand break site for repair.

To specifically promote this repair pathway, fusion proteins consisting of Cas endonucleases and 5'-3' exonucleases are under development. These exonucleases support end resection and actively prevent competing repair pathways, such as non-homologous end joining (NHEJ).

We adapted previously described fusions of Cas9 and plant or viral exonucleases. Furthermore, a literature research identified the Mre11-Rad50-Nbs1 (MRN) complex as a suitable candidate for this purpose. This enzyme complex generally plays a central role in recognising and processing DNA double strand breaks (DSBs), and thereby selecting the repair pathway. Mre11 is an enzyme with endonuclease and exonuclease activities that plays an important role in starting the process of DNA resection, the prerequisite for MMEJ.

After selection of Mre11 and Rad50 from maize *(Zea mays)* and synthesis of the sequences, initial experiments focused the influence of the linker between Cas9 and Mre11, as little is currently known about this subject. Various linkers were tested, including a medium-rigid linker, a short flexible linker, a linker-free variant, and an XTEN linker. All the variants were tested at four target motifs in barley protoplasts. To evaluate their functionality, we analysed the mutation efficiency and the length of the resulting deletions. The Cas9 fusion to the viral UL12 exonuclease outperformed the fusion to *Arabidopsis thaliana* Exonuclease 1. To connect Cas9 with maize Mre11, the middle rigid linker exhibited higher median values across all target motifs. These results provide initial indications of the effect of the right fusion partner to Cas9 and of the potential influence of linker architecture and form an important basis for further optimisations and experiments.

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Pan-omics insights into vernalization regulation in barley

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The barley pan-epigenome profiles regulatory features from DNA to chromatin in seedling leaves across up to 20 genotypes. When integrated with pangenome and pan-transcriptome datasets, these seedling-stage profiles provide a framework to study key biological processes, including vernalization, in which prolonged cold exposure primes plants to flower. In winter barley, vernalization response is mediated by *VERNALIZATION 1 (VRN1)*, whose upregulation is associated with a shift from repressive (H3K27me3) to activating (H3K4me3) histone marks. In contrast, spring barleys, typically carrying deletions of the flowering repressor *VRN2*, bypass vernalization and exhibit active *VRN1* chromatin states before vernalization. These are evident in typical winter and spring barley genotypes across the barley pan-genome. However, flowering time experiments revealed additional variation: some winter types, termed soft winter, can flower without cold treatment, yet remain vernalization-responsive. While steady state *VRN1* transcript levels before vernalization generally reflect the winter-spring classification, spring barleys exhibit a spectrum of VRN1 expression. At the epigenomic level, soft winter types display intermediate *VRN1* chromatin states, with both H3K4me3 and H3K27me3 present but at lower levels than in spring or strong winter types. Open questions remain regarding the regulatory impact of VRN1 intron 1 length, and the role of retained *VRN2* copies in spring barleys. Together, these pan-omics data highlight the complexity and diversity of vernalization regulation in barley.

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Breeding – mediated phenological shifts and their role in wheat climate adaptation

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Introduction
Climate change poses an escalating threat to wheat production via rising temperatures and variable precipitation (Zhang et al., 2022). Although breeding programs primarily target yield, inadvertent shifts in phenology may have significantly altered crop exposure to environmental stressors. Whether yield-centric selection has fostered "accidental" climate adaptation or heightened vulnerability remains unknown. This study evaluates the impact of German wheat breeding on heat and drought stress exposure across key developmental phases and diverse climatic gradients.

Methods
We evaluated 13 representative German winter wheat cultivars spanning a breeding history from 1895 to 2007, categorized into historical (1895-1949) and modern (1961-2007) eras. A process-based phenological model, grounded in photo-vernal-thermal responses, was parameterized using field data from western and central Germany. This model was scaled across 127107 grids (1km×1km) for the period 1960-2021. Exposure to heat stress and drought was quantified for both vegetative and reproductive phases. Additionally, we simulated multiple sowing scenarios (Sept 15 – Nov 15) to evaluate management flexibility. Statistical significance and regional variations were determined through temporal trend analysis, spatial mapping, and Cliff's Delta effect size quantification.

Results
Modern cultivars significantly reducing vegetative heat stress (22%) by shortening pre-flowering development by approximately 5 days. During the reproductive phase, phenological repositioning extended grain-filling duration by 2 days. This prevented expected heat intensification, achieving a 5% reduction in heat exposure despite the longer sensitive window. Breeding-driven shifts also redistributed 4.4 mm of precipitation from the vegetative to the reproductive phase, although modern cultivars faced a marginal increase in dry-day frequency. Yet both breeding eras showed parallel increases in reproductive heat exposure (2.34-2.37°C days per decade), modern cultivars exhibited 9% lower intensity at the extreme 31°C threshold. Finally, sowing date flexibility proved limited as delaying sowing by 42 days compressed vegetative growth but minimally impacted reproductive duration (1.4-1.9 days), while systematically increasing reproductive temperatures (0.5-0.7°C) and heat intensity (1.5-1.9°C days) without substantial moisture benefits.

Conclusion
Historical breeding for yield has accidentally optimized German wheat phenology, reduced vegetative heat stress and shifting reproductive phases toward more favorable precipitation windows. Despite these gains, the increasing frequency of extreme heat and the limited efficacy of management adjustments like sowing date shifts highlight a critical gap. These findings underscore that while phenological auto-adaptation has provided a historical buffer, future climate resilience will require integrating specific physiological stress-tolerance traits alongside continued developmental optimization.

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Microbiome – Enabled Adaptation of Wheat to Nitrogen Deficiency and Drought

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Sustainable crop production under increasing nitrogen limitation and drought stress represents a central challenge for future agriculture. While crop genetic adaptation and microbial interaction has achieved substantial progress (Yu et al., 2021; He et al., 2024, 2025; Li et al., 2026), the potential of the wheat-associated microbiome to enhance stress resilience and yield stability remains insufficiently explored, particularly across diverse genotypes and environments. This subproject of DRIVE aims to decipher how wheat–microbiome interactions contribute to adaptation under nitrogen deficiency and drought, and how these interactions can be harnessed for future-ready cropping systems.

Integrative Multi-Omics Analysis Across Wheat Genotypes

We investigated 17 genetically diverse wheat genotypes grown under controlled nitrogen deficiency and drought conditions. Using rhizosphere 16S rRNA gene sequencing in combination with root transcriptome profiling, we captured genotype-specific and stress-responsive microbial assemblages alongside host gene expression programs. Integrative analyses revealed distinct microbial taxa and interaction networks associated with nitrogen stress responses, water limitation, and stress-adaptive root traits. These taxa were consistently linked to transcriptional signatures related to nutrient uptake, hormone signaling, and abiotic stress tolerance.

From Correlation to Function: Cultivation and Validation

To move beyond correlative patterns, we isolated key bacterial taxa identified through integrative analyses and established a targeted cultivation platform. Selected strains were functionally validated for their capacity (i.e. auxin production, nutrient mobilization and biofilm formation) to enhance drought resilience and performance in wheat under greenhouse conditions and subsequently under field trials. These experiments confirmed that specific microbial taxa can reproducibly improve plant performance under abiotic stresses, providing a functional bridge between multi-omics discovery and agronomic relevance.

Heritability, Environmental Shaping, and SynCom Design

Future work will explore whether and how beneficial microbial taxa can be transmitted across plant generations, addressing the heritability and stability of stress-adaptive microbiomes. In parallel, multi-year and multi-site field experiments will assess how environmental and climatic variability shape wheat-associated microbiomes across soils, seasons, and management regimes. Ultimately, we aim to construct synthetic microbial communities (SynComs) that establish stable, functional consortia capable of promoting yield and stress tolerance under low-input and climate-challenged conditions.

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Enhancing local adaptation by introducing new traits through genome editing

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With ever-increasing efficiency, big data and other approaches can identify a large number of potentially useful alleles that hold great promise for targeted plant breeding. However, these new alleles are often found in old, low-yielding landraces or even wild relatives. Introducing them into elite cultivars through conventional crosses comes at a high cost, as yield and other desirable traits are compromised by carrying many suboptimal alleles at other loci. To remedy this, several rounds of backcrossing to the elite parent are required, which is time consuming and labor intensive. And even then, the end result may still be affected by unavoidable residual linkage drag. The problem is compounded when several of these promising alleles from different sources need to be stacked.

Modern genome editing technologies, mainly developed in the last decade, now offer an easy way out of this conundrum. CRISPR/Cas9-based targeted DNA base editors and similar tools can introduce the desired changes directly into the elite varieties without causing any other alteration in the genome. Stacking is not a problem, as multiple targeted mutations can easily be introduced in parallel. Not even a transgene encoding the base editor will be left after successful editing, for example by segregation after simple selfing or outcrossing, making the resulting plant essentially non-GMO (if the expected changes in EU legislation take place).

While the main focus of the DRIVE project is to identify alleles that will enable wheat to thrive under the future climate conditions expected for Germany around 2050, our work package will initially focus on alleles that confer resistance to yellow rust. Such alleles have been identified, for example, in the Genbank2.0/3.0 project.

Wheat elite cultivars are notoriously recalcitrant to attempts at genetic transformation. However, recent studies (Debernardi et al. 2020) have suggested that using genes for certain developmental regulators like GRF-GIF chimeras could help to overcome this hurdle. We are therefore currently establishing a transformation and genome editing pipeline for elite winter wheat cultivars in our hands, to later introduce candidate alleles for yellow rust resistance and then evaluate the performance of the genome-edited plants when challenged with the fungal pathogen.

The initial results with the “Partner” variety are very promising and should enable us to introduce the first transgenes with agricultural potential in the near future. We also have other wheat varieties in the testing pipeline, such as “Benchmark”.

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Weather-driven responses of wheat genotypes across Germany

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Introduction
Globally, wheat production faces growing risks from climate change due to rising temperature and declined precipitation (Abdelrahman et al., 2020). Europe is not an exception. Across Europe, current wheat genotypes are projected to gain 4% with fertilization, versus a 9% decline without it (Webber et al., 2018). Under breeding programs, identifying locally adapted wheat genotypes requires a systematic evaluation of each cultivar's response to climatic extremes. This is crucial for determining thresholds and uncovering the mechanisms underlying yield variability. This study investigates the influence of precipitation- and temperature-based indices at different phenological stages on wheat production in Germany.

Methods
Over 220 wheat genotypes from the BRIWECS dataset were evaluated at five locations across Germany between 2015 and 2019 (Sabir et al., 2023). The genotypes were classified by phenology and height into four groups. These included: early-tall, early-short, late-tall, and late-short. Six weather indices were derived from precipitation and temperature—including heat intensity, heat frequency, duration of hot days (temperature-based), and intensity, frequency, and duration of dry periods (precipitation-based) (Becker et al., 2025). The indices were calculated during the vegetative and reproductive phases to assess their relationships with yield components. These relationships were analyzed using a random forest model, and the contributions of each index to yield variations were quantified via SHAPLEY analysis.

Results
Preliminary results showed that the intensity of dry periods was overall the most influential variable across all cultivars. However, the relative importance of indices varied among different cultivars. For late-short cultivars, the number of dry days from sowing to flowering was identified as the most important resulting by up to 14% in yield reduction. For early-tall cultivars, the intensity of dry period followed by the intensity of hot days during reproductive phases were identified as most important proxies.

Conclusion
We found that our machine learning approach could effectively extract signals from weather indices, providing a valuable framework for assessing the responses of different wheat cultivars to drought and heat stress. Our approach enables the identification of key climatic drivers affecting yield and can support breeding programs by highlighting cultivar-specific sensitivities to extreme weather events.

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Revisiting self-incompatibility in rye – the S locus genomic region

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FSelf-incompatibility (SI) in rye *(Secale cereale L.)* is a bifactorial genetic mechanism that promotes outcrossing and has long been recognized as a key determinant for maintaining genetic diversity and contributing to population resilience under variable environmental conditions. While comparative genomics facilitated precise mapping of the Z locus on rye chromosome 2R two decades ago (Hackauf and Wehling 2005), the broader genomic footprint of variation at the S locus on chromosome 1R has remained largely unexplored.

We generated genome-wide SNP profiles in a biparental population segregating for a self-fertility mutation at the S locus using a custom 25k+5k wheat/rye SNP array. Genome-wide segregation analysis revealed pronounced transmission ratio distortion in the F_2 population, with the strongest effect localized to the S locus region on chromosome 1R. Beyond this region, additional segregation distortion loci were detected on chromosomes 2R–6R, suggesting a broader genomic footprint associated with S locus variation.

We retrieved coding sequences based on gene models from the Lo7_v2 genome assembly (Rabanus-Wallace et al. 2021) and orthologs were identified across 16 additional monocot species representing major Poaceae lineages and related taxa. Codon-aligned multi-species sequences were analysed using likelihood ratio tests implemented in PAML. Preliminary multi-species codon-based analyses indicate that signatures of positive selection are confined to two DUF247-annotated transmembrane proteins at the Z locus on chromosome 2R, consistent with their proposed role in pollen–stigma recognition processes (Melonek et al. 2021). In contrast, candidate genes within the 1R S locus region did not show comparable signals under the applied site-model framework. Further analyses accounting for taxon sampling and alignment stringency will refine these initial observations.

Integration of defined S and Z locus genotypes into rye pangenomic frameworks will enable the separation of locus-specific and epistatic effects on fertility and segregation patterns, thereby translating genomic insights into predictive markers for controlled crossing and hybrid breeding.

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Rye Pangenomics – Then, Now, and Beyond

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Rye (Secale cereale L.) is a high-yielding, stress tolerant cereal crop whose improvement is hampered by its large, repeat-rich, and highly heterozygous genome. Particularly, in outcrossing landraces and plant genetic resources (PGRs), the genomic complexity challenges genome assembly and limits the exploration of rye's genetic diversity. This creates an urgent need for a highly homozygous, chromosome-scale reference genome to serve as a foundation for research, as well as a phased assembly pipeline capable of resolving heterozygous regions in PGRs and capturing the full genomic diversity of rye for genetic studies and genome-based hybrid breeding.

Previous rye genome assemblies were limited by incomplete centromere resolution, misoriented scaffolds, and fragmented repetitive regions, restricting their utility for detailed genomic and breeding studies [1-3]. To overcome these limitations, we generated Lo7_V3 [4], a high-quality, chromosome-scale assembly of the highly homozygous inbred line Lo7, generated using PacBio HiFi, Oxford Nanopore, Hi-C, and BioNano data via the TRITEX pipeline. The assembly spans 6.76 Gb with a contig N50 of 128 Mb, corrects previous misorientations, anchors previously unassigned sequence to the seven pseudomolecules, and achieves complete centromere assemblies across all seven chromosomes.

To address the extensive heterozygosity of the rye genome, here we developed RyePhase, a phased genome assembly pipeline enabling haplotype-resolved genome reconstruction. We applied this pipeline to a F1 hybrid between inbred lines Lo7 and Lo225, a PGR × Lo7 F1 (RYE-HUB26), and the accession R767 (Secale cereale subsp. ancestrale). This approach resolves heterozygous regions, detects structural variants, and reconstructs multiple haplotypes, providing a robust framework for exploring allelic diversity across rye accessions.

Building on these resources, we aim to construct a comprehensive rye pangenome by integrating additional landrace germplasm through the RYE-HUB project. Consistent with rye's obligate outcrossing reproduction system, this framework will enable systematic exploration of genomic diversity across heterotic elite breeding pools and rye genetic resources, including variation at key reproductive loci, copy number variation, disease-resistance loci (NLRs), and centromere dynamics.

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An exploratory pilot study of the early-stage root architecture of rye commercial hybrids and landraces

L. Weiler-Tersch[1], F. Fiorani[1], B.Stich[2], B.Hackauf[2], D. Siekmann[3]

Previous studies of selected rye germplasm (Secale cereale) indicated that the species has a good tolerance to abiotic stresses such as drought. In addition, rye has been cultivated since centuries on marginal soils with poor nutrient availability. These attributes make it a very good candidate for climate resilient and low input agriculture. While these favourable traits are present in both natural variants and commercial hybrids. It is likely that several of these traits depend on the development of a vigorous root system. However, detailed knowledge regarding rye root system architecture and its development is currently incomplete. This pilot study aimed to preliminary assess whether modern breeding for improved aboveground traits had an impact on the early-stage root system of rye. We hypothesized that the root system of rye varies between landraces and commercial hybrids. Therefore, we evaluated two commercial hybrids and two German landraces ('Petkuser Kurzstroh' and 'Mecklenburger Marien'), with the latter specifically selected to represent the two major heterotic gene pools in rye breeding, Petkus and Carsten. Plants were grown in soil-filled rhizotrons for an 18-day growth period under controlled greenhouse conditions. Utilizing the high-throughput, non-invasive root phenotyping platform GROWSCREEN-RhizoIII, the temporal development of belowground traits including total root length, root system width, convex hull area and lateral root length was quantified daily. In parallel leaf area and shoot development were measured throughout the experimental period. First, we could not detect significant differences of shoot traits between commercial hybrids and landraces. Further, and partly contrary to our hypothesis, root traits such as total root length, system width, and depth remained comparable across all genotypes. However, we found a significant difference in the spatial distribution of lateral roots along the depth of the rhizoboxes among some of the genotypes studied. Specifically, the landrace 'Petkuser Kurzstroh' allocated approximately a higher proportion of lateral roots to the upper soil layers, whereas the other genotypes produced fewer lateral roots in the same region. Conversely, the hybrid 'SU Arvid' concentrated the majority of its lateral roots at a depth of approximately 40 cm. Finally, we found a clear difference in the stability of trait expression. Hybrids exhibited a higher degree of homogeneity, whereas landraces displayed a larger intra-genotype variation. As landraces represent heterogeneous populations of highly heterozygous individuals, these results align with biological expectations regarding their population structure. In conclusion, our results suggest that within the specific developmental window studied and under the conditions of this pilot study, modern hybrid breeding may have not fundamentally altered early-stage root system architecture in rye. Comparing a larger number of hybrids and landraces is required to generalize these results. However, the high degree of individual variation found within landraces highlights their value as diverse genetic reservoirs. Future research should investigate in detail the extent of root phenotypic diversity of landraces potentially linked to distinct survival advantages under environmental stress.

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Accelerating Doubled Haploid Production in Rye Through Chemically Induced Microspore Reprogramming

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Introduction & Objectives The development of doubled haploid (DH) lines is a cornerstone of modern breeding, enabling the generation of homozygous plants in a single generation. While enabled in crops like wheat and barley, rye *(Secale cereale)* remains recalcitrant. Within the RYEHUB project, ScreenSYS applies innovative solutions to enable efficient *in vitro* reprogramming of rye microspores.

Optimizing Donor Material & Isolation A critical determinant of success is the physiological state of the precursor cells. We defined the boot stage as the optimal harvest developmental time. Isolation and purification protocols were developed, routinely yielding microspore populations with 70% viability.

Media Engineering & Viability Maintenance Using a high-content screening platform, we tested 23 distinct medium formulations. According to viability kinetics and microspore morphology assessment, the medium SM8.13 was selected for the initial SOP version. In this formulation, viability loss was limited to ~50% after 14 days—a significant improvement over traditional basal media. This enhanced cellular integrity is a fundamental prerequisite for the subsequent induction of sustained cell divisions.

Induction of Microspore-Derived Callus/Structures (MCS) Initial microspore proliferations were observed in SM8.13 when enriched with a specific supplement. This synergistic effect has paved the way for high-frequency formation of microspore-derived multicellular structures (MCS). Current results indicate that the protocol is now both reproducible and robust.

Future Directions & Chemical Reprogramming The next phase focuses on intensifying proliferation rates. ScreenSYS will utilize its library of >250,000 compounds to identify novel, rye-specific regulators including recently identified proprietory compounds with proven effect in other crops.

Upcoming tests aim to enable MCS outgrowth following plant regeneration to complete the pipeline from haploid cell to fertile DH plant.

Keywords:
Secale cereale, Doubled Haploids, Microspore Culture, Culture Medium, MCS Induction, Chemical Screening, Plant Breeding.

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The rye nested association mapping population – a resource for the Triticeae genetics community

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In rye *(Secale cereale L.)*, hybrid breeding increases and secures sustainable grain production on finite arable land without increasing water and fertilizer use. This is presumably one of the reasons compared to wheat, barley and even oat, there is no sizeable rye research community and no consistent development and use of common genetic resources. As public immortal mapping populations are not available, mainly elite rye germplasm from Germany served for rye QTL mapping (cf. Hackauf et al. 2022). A large set of rye recombinant inbred lines (RIL) in the public domain would allow a wide range of scientists to integrate their research together in community efforts and community databases (e.g. GRAMENE) and unravel additional scientific findings.

We adapt here the strategy of Nested Association Mapping (NAM) for complex trait dissection in rye. The key feature of NAM is the possibility of joint-linkage and association mapping exploiting genotypic and phenotypic data in a large population of RIL for investigating quantitative traits and associated genomic regions leading to rapid discovery of candidate genes and markers. We have started to develop a large-scale rye NAM population (JOSY = JKI Open Source Rye), and established 5,489 RILs derived from the crosses of the sequenced inbred line Lo7 (Rabanus-Wallace et al. 2021) as a common parent with individual plants from each of 68 diverse populations.

The experimental design of JOSY enables to (1) capture rye genetic diversity assessed using the custom 25k+5k wheat rye SNP-array, (2) exploit ancestral recombination, (3) generate germplasm that can be evaluated for agronomic traits at field locations of temperate regions, (4) efficiently take advantage of next-generation sequencing technologies through genetic design, (5) develop a mapping population that has sufficient power to detect species-specific QTL and resolve them to a level of individual genes, and (6) provide a community resource. Family sizes of 100 RILs will allow to identify associations with rare alleles within individual families. The diversity among the parental genotypes increases the likelihood that multiple populations segregate for a given trait or allele, allowing mapping within several bi-parental populations rather than a single, moderately sized bi-parental population. Our design increases the number of alleles to a maximum of 137 and enables improved estimates of genetic architecture and QTL variance as compared to bi-parental linkage mapping or GWAS.

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A physical map of the Ddw1 locus in rye

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The dominant dwarfing gene *Ddw1* is a major factor controlling plant architecture in rye and a novel component in hybrid breeding strategies. As a GA-sensitive regulator of stem elongation, *Ddw1* enables the development of semidwarf hybrid ideotypes with increased lodging resistance and improved yield stability under variable environmental conditions (Hackauf et al. 2022)

To facilitate precise genomic characterization and support marker-assisted selection, we generated a chromosome-scale genome assembly of the Ddw1 inbred line L1591. High-fidelity (HiFi) sequencing produced 240 Gb of long-read data (~36× coverage), with read N50 values between 19.7 and 26.3 kb. GenomeScope analysis estimated a genome size of 6.73 Gb and low heterozygosity (0.05%), confirming a highly homozygous background suitable for reference-quality assembly.

In addition, 612 million Hi-C read pairs were generated, providing substantially higher chromatin contact depth than previously available rye assemblies and enabling high-confidence chromosome-scale scaffolding. The resulting L1591 v1 assembly spans 6.90 Gb, with 6.74 Gb anchored to chromosomes 1R–7R and chromosome-level N50 values ranging from 62 to 133 Mb.

A high-resolution genetic map was constructed to precisely anchor the *Ddw1* locus within the assembled genome sequence, establishing a framework for positional cloning and the systematic development of diagnostic markers for breeding applications. The assembly and high-resolution integration of *Ddw1* successfully established the genomic framework for ongoing functional genomic analyses of the locus.

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From Diversity to Resilience – Revisiting Leaf Rust Resistance for Tomorrow's Semi-Dwarf Rye

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Hybrid rye *(Secale cereale)* breeding has successfully addressed the challenges of rye's unique reproductive biology through the development of high-yielding cultivars with improved resource-use efficiency. Current breeding efforts increasingly focus on semi-dwarf ideotypes with modified plant architecture to improve lodging resistance and yield stability. These architectural changes (including the hypothesized elevating relevance of the flag leaf as photosynthetic organ) are expected to further increase the importance of durable leaf health, particularly with respect to leaf rust *(Puccinia recondita)*. This issue is further compounded by a relatively low leaf rust resistance level observed in modern cultivars attributed to the lack of resistance genes within the original hybrid gene pools Petkus and Carsten.

To re-explore available resistance diversity, a large-scale screening of rye genetic resources was initiated using a representative contemporary *P. recondita* population. High-throughput phenotyping was achieved by combining the Macrobot platform with automated image analysis to quantitatively score infection levels on detached leaf segments. Released hybrids representing all three major rye breeding programs were systematically evaluated, thereby providing a benchmark for the current level of leaf rust resistance in elite breeding material. Comparisons between seedling assays and young vernalized plants showed moderate correlations (r = 0.38), indicating largely consistent resistance patterns across early developmental stages. An independent repetition of the inoculation experiment with hybrids resulted in a moderate correlation (r = 0.42) as well.

First results from more than 660 gene bank accessions confirmed the generally low resistance level across rye germplasm but revealed pronounced quantitative differences among specific populations. In fewer than five populations, genotypes with a clear hypersensitive response and absent uredinia formation were identified. Resistant populations showed infection scores approximately tenfold lower than the susceptible standard. Linking passport data with preliminary results enabled a prioritized selection of accessions for subsequent screening of extended germplasms.

Identified resistance donor candidates will be validated and introgressed into well-characterized genetic backgrounds, including semi-dwarf breeding material. Genome-wide association studies based on high-density marker data will be conducted to map underlying resistance loci, followed by field validation under contrasting canopy architectures.

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Effects of the Ddw1 Semi-Dwarfing Gene on Root Architecture and Drought Response in Winter Rye

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RYESILIENCE project responds to the increasing need for drought-resistant crop varieties to ensure food production under climate change. This project aims to assess the effects of the GA-sensitive semi-dwarfing gene *Ddw1* on root system architecture and agronomic performance across developmental stages in winter rye. Although winter rye is well known for its adaptation to hard soils and adverse climatic conditions, the role of *Ddw1* under drought conditions remains largely unexplored (Hackauf et al., 2022). Currently, our priority is to determine whether Ddw1 modifies root system architecture and drought responses in winter rye without compromising early establishment.

The project examines drought stress at different developmental stages, through winter rye evaluations from more controlled environments (rhizotrons) to field trials. So far, we have set up a rhizotron to study roots before tillering (Kirchgesser et al., 2023) and a field trial during stem elongation and heading to assess root growth and plant performance. Pre-tillering assessments showed that semi-dwarf genotypes consistently produced lower shoot biomass, resulting in a higher root-to-shoot ratio during early growth. In contrast, no significant differences were observed in root length, root biomass, or root tip number between semi-dwarf and tall varieties. Together, these findings suggest that *Ddw1* does not negatively affect early root growth while altering biomass allocation in ways that may influence later drought responses. In addition, a rainout shelter experiment evaluates drought stress during stem elongation and heading. This experiment is still ongoing, and we aim to assess root performance, canopy temperature, and productivity.

Our results indicate that the *Ddw1* gene does not negatively affect early root development, while suggesting that genotypic differences may emerge at later growth stages. These findings suggest that Ddw1 represents a promising genetic resource for improving drought tolerance in winter rye without compromising early plant establishment. *Ddw1* appears compatible with early root establishment while offering potential advantages under later drought stress.

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Functional characterization of Flavonoid UDP-Glycosyltransferases in Brassica napus

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Flavonoids are a large class of phytochemicals that exhibit various bioactivities in nature. They contribute to plant growth, development, reactions to environmental stresses as well as defense against plant pests. Therefore, they are an important target for plant breeding. Additionally, their repertoire of diverse bioactivities is of great commercial interest for biotechnology with potential implications for prevention and treatment of various human diseases. Among other modifications, glycosylation of Flavonoids by UDP-glycosyltransferases (UGTs) is one important reason for the vast diversity of Flavonoids found in plants. In rapeseed *(Brassica napus)* multiple Flavonol glycosides, including Kaempferol- and Quercetin derivatives, have been identified and associated with important functions for plant defense1. Their bitter off-taste however, limits the utilization of rapeseed protein as a sustainable and high-quality, plant-derived protein source for human nutrition2. While many key enzymes for Flavonoid aglycone biosythesis in B. napus have been identified and characterized previously, the exact functions and specificities of many members of the extensive UGT enzyme family are still largely unknown. Within this work we identify3 and investigate selected *B. napus* UGTs to further characterize the Flavonoid glycosylation *in vitro* and *in vivo*. By focusing on UGTs with main activity in seeds, we aim to advance breeding strategies to improve the taste quality of rapeseed protein.

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RaPEQ 3 – Phenomic Selection for Protein Yield in Hybrid Rapeseed – Model Optimization and Early Prediction Using F1 Seed NIRS

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Increasing the domestic production of high-quality plant protein is a central objective in European agricultural production. . Although oilseed rape represents one of the most productive protein crops in temperate regions, breeding efforts have traditionally focused on oil content, leaving protein yield comparatively underexplored. Phenomic selection based on near-infrared spectroscopy (NIRS) offers a cost-efficient and high-throughput approach for predicting complex traits such as protein yield in large breeding populations.

Within the RaPEQ3 project, we evaluated and optimized NIRS-based phenomic prediction models for protein yield in winter oilseed rape F1-hybrids. The dataset comprised 120 experimental oilseed rape hybrids tested across four field locations, complemented by their parental lines grown at one location. In addition to predict hybrid performance based on parental NIRS data, we implemented an innovative approach in which NIRS spectra collected from F1 hybrid seed prior to sowing were used to predict protein content and protein yield of the harvested hybrids.

Predictions were performed using a mixed-model framework, with particular emphasis on optimizing preprocessing of spectral data. Bayesian optimization was applied to systematically tune preprocessing parameters, allowing efficient exploration of the parameter space and identification of configurations that maximize the prediction accuracy. Different training–validation strategies were evaluated to reflect practical breeding scenarios, including cross-environment predictions.

Our analyses indicate that optimized models enable reliable prediction of protein yield in oilseed rape hybrids based on NIRS spectra collected before sowing. The integration of spectral information from parental lines and pre-sowing F1-hybrid seed material could enhance the potential for early selection decisions for the development of high-protein rapeseed varieties in future hybrid breeding programs.

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WP2 – Sclerotinia sclerotiorum on quinoa – disease epidemiology and pathogenicity

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Quinoa is a promising crop with high nutritional value and resilience to various biotic and abiotic stresses, offering potential to diversify and strengthen Central European agriculture. Despite this, its adoption is limited by suboptimal adaptation to local environments and susceptibility to certain pathogens and insect pests. The 'Quinoa for Future Diversified Agricultural Systems (Q4F)' project addresses these limitations by integrating genomics, high-throughput phenotyping, and crop modeling to define ideotypes optimized for Central European cultivation, enabling the sustainable improvement of quinoa for regional agricultural systems.

The objective of WP2 is to elucidate the epidemiology, pathogenicity, and genetic determinants of resistance to the necrotrophic fungus *Sclerotinia sclerotiorum* in quinoa. This pathogen poses a significant threat by causing yield losses and exhibiting genotype-dependent variation in virulence. To this end, a globally representative panel of 300 quinoa accessions will be evaluated using high-throughput detached-stem infection assays. Time-lapse imaging system will be used to monitor disease progression at high temporal resolution, focusing on the *S. sclerotiorum* 1980 reference isolate.

Preliminary experiments on 20 accessions have confirmed feasibility and informed the design of the full panel. Genome-wide association studies (GWAS) will identify resistance-associated loci, while transcriptomic profiling on selected genotypes across defined infection stages will uncover the molecular mechanisms underlying resistance and pathogen virulence. Weighted gene co-expression and gene regulatory network analyses will be applied to both plant and pathogen to identify key resistance regulators in quinoa and virulence determinants in *S. sclerotiorum*.

Population genomic analyses of 61 *S. sclerotiorum* isolates collected over three years from multiple German field sites will allow to assess genetic diversity, population structure, and variation in virulence-associated genomic features of the pathogen and allow additional screening with highly diverse pathogen isolates.. This analysis will provide insights into the robustness of quinoa resistance under field-relevant conditions.

By integrating phenotypic, genomic, and transcriptomic data across host and pathogen, WP2 will provide a comprehensive understanding of host–pathogen interactions and the genetic basis of disease resistance. The resulting resistance scores, candidate genes, and molecular markers will directly inform genomics-based breeding strategies and support the development of resilient quinoa ideotypes adapted to diverse German growing conditions.

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Process-Based Crop Modelling to Advance Quinoa Breeding and Cultivation in Diversified Future Agricultural Systems

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Background

Climate change, biodiversity loss, and soil degradation increasingly threaten the resilience of European agriculture (Krause et al., 2024). Diversifying cropping systems with underutilized, stress-tolerant species is a promising strategy to address these challenges. Quinoa (Chenopodium quinoa Willd.) offers significant potential due to its high nutritional value and tolerance to drought, salinity, and poor soils (Jensen et al., 2000). Despite growing interest, quinoa cultivation in Europe remains limited by poor local adaptation, yield instability, and susceptibility to pests and diseases (Jacobsen, 2017). The absence of regionally adapted cultivars and tailored agronomic knowledge further constrains its adoption. The Q4F project addresses these gaps through an interdisciplinary approach combining genomics, phenotyping, plant protection, and crop modelling. Its goal is to develop quinoa ideotypes adapted to Central European conditions, with improved yield stability and biotic stress resistance. By delivering genomic markers, phenotyping tools, and model-based ideotype definitions, Q4F supports rapid breeding and integration of quinoa into climate-resilient, diversified cropping systems.

Materials and Methods

The Q4F project evaluates a diverse quinoa panel (>600 genotypes), including genebank accessions, biparental RILs, and European cultivars. Multi-location field trials are conducted in contrasting environments across Germany including Kiel, Berlin, Göttingen and Stuttgart, supported by image-based high-throughput phenotyping and controlled environment experiments. Morphological, physiological and phenological traits (e.g., flowering time, yield components, canopy architecture) are recorded manually and via UAV-based multispectral Indices (e.g., NDVI, NDRE, SAVI). Climate chamber experiments provide crucial data on temperature response as well as photoperiod response as a basis for crop model parameterization. The CROPGRO-Quinoa model within DSSAT (Präger et al., 2019) is used to simulate genotype × environment × management (G×E×M) interactions. Model calibration follows a structured approach using field-derived data on phenology, biomass partitioning, and radiation use efficiency. Gridded (1 × 1 km²) DWD weather and BüK250 soil data serve as environmental inputs for large scale simulation studies. A key organizational element is the coordination between work packages and partner institutes, enabling shared use of genotypes, environmental data, and model outputs across disciplines. A thorough research data management plan ensures standardized and FAIR management of research data along its data life cycle. Based on multi-environment in-situ data and complemented with model-based insights envirotyping and multivariate clustering is applied to classify experimental environments into Target Population of Environments (TPEs). These guide model-based ideotype simulations under current and projected climatic conditions.

Results and discussion

The extended modelling framework is designed to evaluate genotype performance under a wide range of scenarios. It supports the definition of ideotypes that express stable yield, early maturity, and stress resilience across Germany's agroecological diversity. Simulated outcomes provide recommendations for sowing windows, genotype deployment, and management guidelines under both current and projected climate.

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Establishing a phenotyping pipeline for epidermal bladder cells (EBCs) in quinoa

Q.C. Burandt[1], M. Rostás[1], Q.C. Burandt[2]

Quinoa (Chenopodium quinoa) is traditionally grown in the Andean region, especially Peru and Bolivia. Over the past years, global demand for quinoa increased due to its high nutritional value. As a consequence, quinoa production increased both inside and outside its origin region. Quinoa is regarded as a promising candidate for crop production in Germany and similar temperate European countries. However, limited adaptation to temperate climatic and photoperiodic conditions as well as a not fully integrated supply chain currently restrict cultivation on a larger scale. In addition, farmers report problems with herbivores, especially aphids, Lygus spp. and Cassida nebulosa. Today, about 60 farmers grow quinoa in Germany. Leaves and stem of quinoa are covered by highly vacuolated cells called epidermal bladder cells (EBCs). The function of EBCs has not yet been fully explained. They have been proposed to be involved in abiotic stress resistance, including salinity, drought, radiation and cold stress. However, a recent study by Moog et al. 2023 raises doubts and suggests a physical and chemical protective function against herbivores. In subproject E of the BMFTR-funded project “Quinoa for future diversified agricultural systems (Q4F)” we pursue this hypothesis further by assessing the relationship between EBC-related traits, such as EBC size, density and color, and resistance to the aforementioned agriculturally relevant insects. For this purpose, our first goal is to establish a phenotyping pipeline to efficiently phenotype EBC-related traits using a handheld microscope and publicly available deep learning methods for object detection and instance segmentation. Once the pipeline is successfully established, it will be used in various experiments over the course of the project to generate data on EBC-related traits. Combined with data on insect damage, this allows us to assess whether and how both trait complexes are connected. In a first greenhouse trial, leaf images of 222 quinoa accessions were taken using a hand-held microscope. The data will be used for model training and to provide first insights into the genetic variation of EBC-related traits in a diverse set of quinoa accessions.

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Genetic and environmental determinants of hop metabolite formation and their influence on the sensory quality of non alcoholic beers

T. Becker[1], C. Neugrodda[1], F. Schüll[2], J. Stampfl[2]

The HOPTIMIZE project implements genome-enabled breeding in hops *(Humulus lupulus* L.*)* to enable early prediction of yield stability under drought and of product-quality traits relevant to diverse end uses. Beer is the best known product made with hops, whose bitter acids, aroma oils, and polyphenols are essential components of beer flavour—especially as the demand for non alcoholic beers continues to grow. Drought and heat stress have been shown to affect key quality determining hop compounds to varying degrees. However, the impact of these altered metabolomic profiles on the sensory quality of beer remains largely unknown.

WP2 investigates how genetic variation and drought stress during hop cultivation influence the formation of key hop compounds and how these changes affect the sensory quality of the final product, represented here by non-alcoholic beers (NABs). Up to 350 genotypes from different locations and treatments in WP1 will be analyzed using modern analytical platforms (GC-MS, HPLC, NIR) to develop a high-throughput method for key hop components. The aims are to identify compounds with strong genotype x environment interactions and to determine whether drought tolerance compromises brewing quality.

This metabolomic phenotyping will be complemented by a sensory evaluation component. Based on genotype and compound profiles, the consortium will select the 200 most diverse genotypes for sensory testing. These genotypes will be used to produce standardized dry hopped NABs. The Rate All That Apply (RATA) method will be used to develop and implement a tasting scheme with the institute's trained panel and the HVG panel; these panels were validated with commercial NABs and preliminary NABs brewed from hand harvested cones of young plants. From the second year onwards, the established RATA method will be used to evaluate hop aroma intensity, mouthfeel, palate fullness, bitterness intensity and quality, drinkability, overall hop impression, and the general sensory quality of the NAB.

Finally, sensory perceptions of the NABs will be statistically correlated with the measured hop compound concentrations to identify key compounds—or compound groups—that positively or negatively affect hop quality under drought conditions.

In 2025, initial trials were conducted to develop analytical methods. The first step was to determine the optimal sample preparation and develop a new GC method for determining the most important aroma components. In the coming months, this GC method will be used to qualitatively evaluate hop aroma. In addition, a NIR method for determining total oils is being developed, which will be calibrated using the GC method.

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Multienvironmental field trials for phenotyping drought adaptation and quality related traits

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The HOPTIMIZE project implements genome-enabled breeding in hops *(Humulus lupulus* L.*)* to enable early prediction of yield stability under drought and of product-quality traits relevant to diverse end uses. A major challenge for hop breeding and genetics is establishing replicated, multi location field trials that permit precise phenotyping in this perennial, trellised crop: vegetative propagation is inefficient and both time and cost intensive, and trials require extensive land and trellis infrastructure.

In Work Package 1, progeny from connected pseudo F2 populations were genotyped for sex determination following Albrecht et al. (2024). Female progenies were vegetatively propagated through successive in vitro culture and shoot and root cutting cycles. Trials were established at three sites across two principal German hop growing regions using an optimized alpha lattice design with partially replicated trial entries. Two historically drought prone sites were designed to receive contrasting treatments—well watered (optimal irrigation) versus drought stressed—beginning in 2026, while a third, high precipitation site (mean annual rainfall >1,000 mm) was included to characterize yield potential under near optimal moisture conditions. The success rate of planting and rootstock development was over 90% at all locations, providing a robust basis for phenotyping mature plants in successive years.

To evaluate whether first season measurements predict mature plant performance, developmental, yield, and cone quality traits were recorded in 2025. From 2026, additional drought adaptation traits will be assessed, enabling development of a whole genome selection scheme for cone yield and quality across contrasting water regimes.

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Genomic Prediction in a vegetatively propagated crop

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The HOPTIMIZE project aims to establish genome-based breeding in hops to enable early prediction of yield stability under drought stress in combination with traits related to product quality for diverse end-use applications. A major challenge in hop breeding and genetic studies is the implementation of efficient field trial designs to warrant precise phenotyping. The designs must accommodate large numbers of genotypes across multiple environments and water treatments while minimizing space requirements.

Within Work Package 3, an optimized experimental design was developed for replicated hop field trials. Alpha-lattice designs with partial replication (p-rep) were implemented to improve precision and resource efficiency. In total, 303 hop clones of a multi-parental half-sib breeding population, along with parental lines and checks, were evaluated. Trials were conducted at three locations: Stadelhof and Zell, each including control and drought stress treatments, and Tettnang with a control treatment only. The experimental layout consisted of blocks with six plots and three plants per plot. Appropriate randomization procedures for the augmented incomplete block design were implemented. Measurements of development, fitness, quality and flowering related traits were taken on first-year emerging hop plants. Data analysis is currently in progress. First results will be used to define maturity groups to organize the harvesting according to the optimal timepoint for each line. The resulting data will enable us to establish a whole genome-based selection scheme for cone yield and quality traits under contrasting water availability.

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Changing vitis leaf hairs by grafting-induced gene editing

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This project aims to restore leaf ribbon trichome density via an innovative CRISPR/Cas gene-editing strategy to ensure climate-resilient viticulture. *Vitis vinifera*, a key economic crop in Germany for winemaking, faces increasing yield losses due to climate change (drought, heat, heavy rain) and especially pathogens like *Plasmopara viticola*, the causal agent of downy mildew. Current control relies heavily on chemical fungicides, necessitating the development of pathogen-resistant cultivars. North American wild species such as *V. labrusca* exhibit dense ribbon trichomes on the underside of the leaves, which build a physical barrier reducing water availability for downy mildew sporangia germination but also minimizes water loss, and shields against UV radiation. However, *V. vinifera* form no or very low numbers of ribbon trichomes. By dissecting the genetic basis of ribbon trichome formation on Vitis leaves and implementation of a transgene-free genome editing pipeline we aim to restoring ribbon trichome formation in *V. vinifera* to enhance abiotic and biotic stress resilience without compromising grape quality. A major challenge lies in the recalcitrance of grapevine to transformation and its genotype-dependence in classical CRIPSR/Cas system approach. A breakthrough approach combines CRISPR/Cas9 with grafting: a Cas9/gRNA-transgenic rootstock is grafted onto a non-transgenic scion. Mobile RNA elements enable transport of Cas9 and gRNA transcripts across the graft junction. This enables targeted genome editing in the scion, yielding heritable, transgene-free edited plants. The combined approaches – identification of trichome forming genes and their precise CRISPR/Cas9-mediated knockout by grafting – aims to increase both climate-resilience and pathogen resistance of V. *vinifera cultivars*.

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Time series analysis in a maize landrace reveals rapid fixation of beneficial alleles

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Identifying genetic loci in the genome that allow a population to respond to selection pressure is essential to understand evolution and improve crops. Temporally consecutive generations under selection offer the opportunity to identify signatures of selection. Maize, as one of the most important crops worldwide is rich in genetic diversity and a model for breeding advances. Therefore, it is an ideal system for studying genetic changes due to selection. Here, we study the genetic changes in two replicates of a selection experiment in a European maize landrace, which showed rapid trait improvement over three cycles of selection. We identified an increase in genetic divergence across successive doubled-haploid populations derived from each selection cycle, consistent with the effect of strong directional selection. However, the genetic divergence observed between the replicates was greater than the one within. In addition to the genome-wide signal, we identified multiple candidate loci under selection through temporal F_{st} outlier analysis comparing the original landrace population to subsequent cycles. These loci showed a significant overlap with regions controlling selected and unselected traits, which were previously identified in a maize landrace genome wide association study. The significant overlap of selected loci shows the importance of major loci in response to directional selection, while the large number of non-overlapping loci demonstrates a polygenic response. Our work shows that the temporal dimension in plant breeding time-series enables the identification of candidate loci under selection and how populations can cope with strong selection through rapid genomic change.

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A Interactive Tomato Pangenome Built From 11 Public Lines Using Pantograph

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Understanding the full spectrum of genetic variation within crop species is essential for trait dissection, and the identification of targets for crop improvement. While traditional analyses focus on single nucleotide variants relative to a single reference genome, pangenome approaches enable the exploration of non-reference sequences and large structural variation that are frequently overlooked but highly relevant for phenotypic diversity.

Here, we present an interactive tomato pangenome generated from 11 publicly available genome assemblies and constructed as a chromosome-level pangenome graph. The pangenome is constructed, visualized and explored using *Pantograph*, an interactive genomic data hub designed to make complex pangenome and multi-omics data accessible without requiring advanced computational expertise.

Pantograph enables the seamless visualization of small- and large-scale genetic variation across multiple zoom levels, ranging from single-nucleotide resolution to whole-chromosome views within a single framework. Genetic variation can be explored alongside multiple genome annotations and arbitrary metadata, such as gene annotations, phenotypic information, or external experimental data. This allows users to inspect candidate regions, highlight structural variation, and associate larger genomic haplotypes with traits of interest, extending beyond classical SNP-centric analyses.

The tomato pangenome of publicly available lines serves as a proof of concept for the InnoTom Project and will be enriched using project specific lines data in the upcoming phases. It provides an interactive entry point for studying the full spectrum of genetic diversity in tomato and illustrates the broader applicability of Pantograph as a comprehensive platform for pangenome-driven research in crop species.

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Identification of genes involved in temperature stress tolerance

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In order to improve the narrow germplasm pool of European Flint in terms of its stress tolerance, we are searching for favourable alleles in landraces that are well adapted to temperate climate. In several cases, it has already been shown that QTLs are the result of changes in transcription of certain genes. Therefore, we wanted to identify maize lines that differ in their transcription under temperature stress. Doubled haploid (DH) lines developed from the European landraces Kemater (from Austria) and Petkuser (from Germany) were grown under controlled temperature conditions and phenotyped. Data on growth rates, plant height, fresh biomass yield, and visible damage were collected at the seedling stage. The most sensitive and tolerant lines were selected for transcriptome analysis of the above-ground parts in v2 seedlings. The transcriptomes were analyzed at five time points: before stress (control experiment), after mild and severe cold stress, after heat stress, and after recovery. For some transcripts, a correlation between cold-dependent expression and phenotype could be demonstrated. To validate the respective candidate genes, the *BonnMU* library was searched for MU-insertion lines for the candidate genes. BC1S1 progeny of the respective lines were phenotyped under cold conditions. It was found that the cold induced phenotype was stably inherited.

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Digital Objects to feature collaborative self-service data management in the INTEGRA project

T. Strauch[1], M. Lange[1], D. Schüler[1], T. Altmann[1]

INTEGRA's integrative data science approach will be supported by state-of-the-art data management efforts devoted to the curation, integration, and harmonization of various datasets from partners' previous projects. This involves connecting public and proprietary data and enabling their combined use, storage, and publication according to FAIR principles (Wilkinson 2016). Data management is co-located with project coordination in WP1 to orchestrate data flows and curation workflows towards AI-ready, integrated FAIR Digital Objects (FDOs). This concept enables the integration of data curation and metadata annotation into an incremental, flexible, and consistent process, known as FAIR by design.

This poster present, how we apply the tooling and infrastructure ecosystem of PLANTdataHUB (Weil 2023), which is based on GitLab and facilitates the collaborative creation of FDOs to produce AI-ready datasets. This ecosystem was developed within the German National Research Data Infrastructure (NFDI), is widely adopted in European infrastructure programs like ELIXIR, and builds upon the expertise of a large number of experts as well as training programs, such as those in the CEPLAS Cluster of Excellence on Plant Sciences.

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WP 3 – Phenotyping of growth dynamics and abiotic stress responses under controlled field-like conditions

R. Shi[1], D. Knoch[1], R.C. Meyer[1], M. Kuhlmann[1], T. Altmann[1]

Project goals

Oilseed rape *(Brassica napus* L.*)* is the world's third-largest source of vegetable oil, and an important resource for the production of protein meal (Tao et al., 2024). Due to its high economic value for both human nutrition and industrial applications, it stands as one of the most important crops in German agroecosystems (Duden et al., 2024). However, increasing climatic challenges, such as flooding, drought, heat waves, and other extreme weather events, have significantly diminished crop yields in recent years (Bathiany et al., 2023; Renziehausen et al., 2025). To accelerate breeding efforts aimed at improving yield stability and productivity, it is crucial to gain a deeper understanding of the physiological and molecular responses to various abiotic stresses, as well as the mechanisms underlying resilience to combined stress factors.

WP 3 contribution

In work package 3 of the INTEGRA-project growth dynamics and abiotic stress responses of oilseed rape plants, including drought, flooding, N-limitation, seed yield and quality are the assessed. The Container/PhenoCrane-system and the Rhizotron system of the IPK PhenoSphere are used, that enables us to simulate controlled, field-like environmental conditions (Heuermann et al., 2023).
In the first experimental season (2025-2026), twelve winter oilseed rape (WOSR) inbred lines are evaluated under four treatments: control, autumn waterlogging (WL), spring–summer drought (DS), and combined waterlogging and drought (CWD). Each genotype is grown in two containers, with 48 plants per container. Throughout the growth period, the containers are imaged using the PhenoCrane multi-sensor platform to assess shoot phenotypes. The plants will be grown to maturity in order to evaluate the effects of these stress treatments on seed yield and quality. In autumn 2025, the waterlogging treatment (triggered by excess watering) was applied to the WL and CWD groups. Under waterlogging, hypoxic conditions caused reduced biomass and reduced photosynthetic performance. Additionally, genotypic variation in response to waterlogging was observed among the lines. Further analyses are ongoing to determine the detailed effects of waterlogging on shoot phenotypes. The impact on seed yield and quality, as well as responses to other stress factors, will be evaluated following harvest in summer 2026 in collaboration with partner IPB.

Root system architecture (RSA) plays a critical role in the response of plants to abiotic stresses (Schneider & Lynch, 2020). To evaluate the RSA of the WOSR lines, preliminary experiments were conducted to assess root growth under drought stress conditions using the automated high-throughput Rhizotron system of the PhenoSphere. Currently, an evaluation experiment is ongoing to assess root phenotypes under two treatments, control and waterlogging, across 18 WORS inbred lines, including the 12 lines of the container experiment. During cultivation, shoot and root images are captured daily to extract traits related to architecture, colour and biomass. This approach will also be used to assess the impact of drought and low nitrogen on root phenotypes in subsequently scheduled experiments.

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WP 1.1 – Project overview and coordination

M. Kuhlmann[1], T. Altmann[1]

Project aims and goals

Yield stability and further increases in productivity and quality of winter oilseed rape (WOSR), the most important oilseed rape crop in Central Europe, are challenged by the increasing frequency and severity of extreme weather events, and reduced fertiliser (N) and pesticide inputs to promote agricultural sustainability. INTEGRA aims to develop and implement a novel integrated breeding technology platform supporting the generation of high-yielding, climate and site-adapted WOSR hybrids with novel functionalities and adaptation to future agricultural practices, simultaneously exhibiting high yield stability and improved product quality. Production of climate-resistant and site-adapted varieties with novel traits requires the consideration of complex genotype-environment (G*E) interactions. Previous data along with new data produced under controlled and field conditions will be used for genomic-phenomic performance prediction, systems biology studies and targeted genetic modification strategies for trait improvement.

Project implementation and coordination

INTEGRA comprises six work packages, which all integrate the two major innovative breeding technologies advanced here, (I) genomic-phenomic prediction of hybrid performance considering G*E interactions and (II) generation of new genetic variants by TILLING and editing of key genes. This combinatorial R&D strategy and the integrative multi-level breeding informatics conducted in WP 2 (provision of plant material and assessment of WOSR hybrid performance in multi-location multi-year field trials including envirotyping and molecular and microbiome analyses), WP 3 (assessment of growth dynamics and abiotic stress responses and resilience mechanisms), WP 4 (assessment of biotic stress responses and resistance mechanisms, and in particular in WP 5 (breeding informatics and DeepLearning) as well as by the JRG AIM4GEM, in which a concerted joint improvement of multiple trait complexes important for climate- and site adaptation is attempted, are unique and highly innovative. Another integrating project component is the assessment of the potential socio-economic and environmental impacts of the achieved WOSR breeding improvements conducted in WP 6, which will also be used as potential corrective to adjust the breeding R&D strategy with its long development periods. WP 1 (project coordination and data management) is carried out at IPK and will use their expertise and longstanding experience in multi-partner, multi-disciplinary project coordination (WP1.1) and in trend-setting data management procedures (WP1.2). The merger of project coordination and FAIR data management into the central management efforts is particularly suitable to support the project strategy of harnessing large amounts of prior data and results of the partners jointly with public data and with complementary newly collected data to an integrated FAIR data space.

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WP 2 – Plant material and assessment of oilseed rape hybrid performance in multi-location multi-year field trials

F. Peleke[1], A. Abbadi[1], S. Rietz[1], C. Flachenecker[2]

Yield stability and further increases in productivity and quality of winter oilseed rape (WOSR), the most important oilseed rape crop in Central Europe, are challenged by changing weather conditions and efforts to increase sustainability in agriculture. INTEGRA aims to support the generation of high-yielding, climate and site-adapted WOSR hybrids with novel functionalities and adaptation to future agricultural practices with reduced fertiliser (N) and pesticide inputs, simultaneously exhibiting high yield stability and improved product quality. The production of climate-resistant and site-adapted novel varieties requires the consideration of complex genotype-environment (G*E) interactions.

WP 2 will generate multi-location phenotypic data, recorded over three cropping seasons. Data will be compiled from field trials using pre-commercial breeding material of genetically diverse rapeseed lines and hybrids. Deep field phenotyping will cover a wide range of traits, including those linked to plant development, yield, seed quality, disease resistance and agronomy. In addition, comprehensive environmental data recording weather and soil conditions will be collected to enable genotype-by-environment (G*E) modelling in INTEGRA. Furthermore, samples from field trials will be made available to monitor rhizosphere microbiomes and plant-associated microbial pathogens, revealing insights into site-specific biotic interactions. Samples from field-grown plants will also be made available to facilitate the generation of transcriptome profiles, enabling the investigation of sources of differential hybrid performance and the identification of differences in regulatory networks across environments. Generated data will be integrated into multimodal INTEGRA analyses to reveal key environmental factors that affect plant performance, as well as the respective genetic plant features and modelling approaches. The status of this project can be seen from this poster.

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Digital Objects to feature collaborative self-service data management in the INTEGRA project

T. Strauch[1], M. Lange[1], D. Schüler[1], T. Altmann[1]

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The poster shows the extension of the framework. We will implement validation jobs to continuously verify the incrementally annotated FDOs against agreed-upon metadata schemas like MIAPPE and Schema.org. To meet agile storage requirements, a Ceph Object Store with an S3 interface is operated to provide high-availability, robust, and scalable storage. In a later project phase, the PLANTdataHUB platform will be extended to streamlined publishing of datasets to suitable repositories, e.g., e!DAL-PGP (Arend 2025).

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AI-Driven Discovery of Gene Regulatory Variants Impacting Rapeseed Performance in INTEGRA

Qinqin Long[1], Jędrzej Jakub Szymański[1]

Our project applies advanced machine learning and network genomics to uncover gene regulatory variants and key control points underlying performance-relevant traits in rapeseed. Large-scale genomic and transcriptomic datasets are integrated within a unified computational framework that links regulatory variation to gene activity and trait prediction.

Gene regulatory and co-expression networks are inferred to identify prime candidate genes and putative master regulators, including transcription factors, based on network topology, centrality, and information flow. These network representations provide a mechanistic context for interpreting complex genotype–expression relationships and prioritizing genes with disproportionate regulatory influence.

Interpretable machine learning models are used to detect informative and predictive features across sequence variation, expression profiles, and network-derived metrics. Feature attribution enables systematic identification of candidate markers and “suspect genes” that consistently contribute to phenotype prediction while ensuring model transparency and robustness.

In parallel, cis-regulatory elements are computationally annotated using sequence-based models and comparative genomics. Regulatory motifs, their genetic variation, and their putative modes of action are inferred through integration with gene expression patterns and regulatory network structure.

Together, this AI-driven genomics approach delivers a scalable strategy for prioritizing regulatory variants, genes, and networks that shape rapeseed performance, providing high-confidence targets for downstream validation within INTEGRA.

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Identification of genomic and transcriptomic features for environment-specific gene regulatory network construction in rapeseed

K. Rockenbach[1], G. Yildiz[1], R. Snowdon X[1], S. Rietz[2], A. Abbadi[2], A. Golicz[3]

Background

Breeding climate resilient crops capable of adapting to varying extreme conditions is an important goal in the face of climate change (Corlouer et al., 2024). Rapeseed *(Brassica napus* L.*)*, Europe's most important oil seed crop, is of particular economic importance. Using genome-wide association studies (GWAS) and transcriptome-wide association studies (TWAS), crop traits can be linked to genomic variants and gene expression, respectively. In the context of the INTEGRA-project (Implementing novel technologies to enhance genetic gain towards resilient rapeseed), we will integrate results from GWAS and TWAS over large sets of genotypes and multiple locations with varying environmental conditions using deep learning to construct environment-specific gene regulatory networks.

Data Collection and Planned Analysis

Long-read sequencing data of 50 elite founder lines from two distinct heterotic pools will be used to call single nucleotide polymorphisms (SNPs) and structural variants (SVs). Based on genomic variations between the founder lines, SVs will be genotyped in an extended set of 300 lines from the same heterotic pools using genomic short-read Illumina sequencing data. SNPs will be called in the 300 lines using the same data. Phenotypic traits of these 300 lines from 7 locations, provided by NPZi, and transcriptomic data collected from 5 diverse locations throughout Germany (Malchow, Hohenlieth: north; Hovedissen: west, Weddegast: east; Moosburg: south) will be used to perform GWAS and TWAS respectively. The transcriptomic data is collected form young leaves using RNA-Seq in 3 successive growing seasons (fall 2024 – spring 2027), pre and post vernalization at each location. An additional set of 500 lines from the same heterotic pools were sequenced at low coverage using short-read Illumina sequencing and will likewise be used to perform GWAS based on SNPs and phenotypic traits at 1 location. In the downstream analysis, the results of the GWAS and TWAS studies will be integrated with environmental data from the various locations in order to link genomic variations to variations in gene expression leading to phenotypic adaptations in response to environmental conditions.

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Composition and structural diversity of microbial communities in the rhizosphere and their effects on the productivity of oilseed rape hybrids

Hendrik Seide[1], Darina Bartels[1], Remco Stam[1], Amine Abbadi[2], Steffen Rietz[2], Daguang Cai[1]

The rhizosphere is a hotspot for a rich diversity of microorganisms, many of which interact directly with the plant in a positive feedback loop, constituting a major element of the plant environment. The rhizosphere microbiome can suppress pathogens and help plants acquire nutrients from the soil (Pineda et al. 2020). Conversely, root exudates strongly influence the rhizosphere microbiome. However, the potential of rhizosphere microbes on plant performance in relation to soil and weather conditions, and the underlying mechanisms, remain largely unexplored and have not yet been considered in OSR breeding and cropping. There is, therefore, great scientific interest and agronomic significance in elucidating the role of rhizosphere microbiome on plant yield in different environments, which is addressed in Work Package Two of the INTEGRA project.

In the first project phase of INTEGA, we focus on surveying the composition and structural diversity of microbial communities in the rhizosphere and understanding their correlation with plant growth, development, defense responses, and seed yields of hybrid varieties in various environments. This survey will provide initial indications, to some extent, of which microbial species or communities at different field trial sites correlate with plant performance parameters. Core sets of the rhizosphere´s microbiome will thereby be identified. These core sets can serve as indicators for soil health for a sustainable hybrid cultivation system and provide a starting point for further research into mechanistic scenarios. This will also facilitate downstream analysis, e.g., to assess the potential of beneficial rhizosphere microbes to maintain or improve soil health and increase the resilience and productivity of oilseed rape. Furthermore, it will enable the screening for genetic variability of responsiveness among project materials, for further improving OSR hybrids.

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Establishment of robust transgenesis and genome editing in oilseed rape (*Brassica napus* L.)

C. Sievering[1], C. Herrmann[1], C. Bollmann[1], B. Rezaeva[1], I. Hoffie[1], J. Kumlehn[1]

Oilseed rape *(Brassica napus* L.*)* is a globally important oilseed crop, whose cultivation potential is increasingly affected by climate change and reduced options of chemical plant protection. Anticipated shifts in climatic conditions and altered pathogen pressure along with predicted limitations in resource availability will negatively impact crop productivity. These emerging challenges highlight the urgent need for modern, sustainable breeding concepts aimed at developing high-yielding, high-quality and resilient oilseed rape varieties. Addressing these challenges requires not only advanced genome editing technology but, as critical precondition, the availability of reliable and widely applicable methods of adventitious shoot formation and genetic transformation.

Within the INTEGRA project, one of the objectives is the establishment and application of genome editing for oilseed rape suitable for an extensive range of genotypes. Initial experiments were focused on the use of hypocotyl explants as starting material for Agrobacterium-mediated transformation. Regeneration and transformation efficiency were assessed using GFP as a reporter, confirming the suitability of the explant type and experimental framework. Building on these encouraging results, systematic optimization of critical parameters – including explant preparation, co-cultivation conditions, and cultivation media – has been initiated. The refined method will subsequently be evaluated across multiple oilseed rape genotypes to assess its reliability and transferability. The establishment of such a broadly applicable method of genome editing will be a fundamental tool beneficial for downstream research and breeding approaches. Selected genes involved in e.g. biotic and abiotic stress responses as well as genes associated with nitrogen use efficiency will be modified using a modular Cas endonuclease-based vector system (CasCADE) with the goal of producing high-performing and more resilient oilseed rape varieties.

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Socio-economic and environmental impact assessment of resilient winter oilseed rape hybrids

H. Kehlenbeck[1], M. Branco[1], J. Saltzmann[1]

Yield stability and further increases in productivity and quality of winter oilseed rape (WOSR), the most important oilseed rape crop in Europe, are challenged by changing weather conditions and efforts to increase sustainability in agriculture. INTEGRA aims to support the generation of high-yielding, climate- and site-adapted WOSR hybrids with novel functionalities and adaptation to future agricultural practices with reduced fertiliser (N) and pesticide inputs, simultaneously exhibiting high yield stability and improved product quality. The production of climate-resistant and site-adapted novel varieties requires the consideration of complex genotype-environment-management (G×E×M) interactions.

To assess the sustainability performance of enhanced WOSR hybrids developed within INTEGRA, an integrated approach combining environmental, economic and societal perspectives is applied. This approach is based on a prospective Life Cycle Sustainability Assessment (pLCSA) framework, enabling the analysis of future-oriented breeding outcomes prior to large-scale adoption. Within this framework, environmental Life Cycle Assessment (LCA) is combined with socio-economic dimensions to capture trade-offs and synergies between productivity, environmental performance and economic viability, explicitly accounting for G×E×M interactions. Key environmental indicators include resource use efficiency, climate change impacts, eutrophication, land use and energy demand. The socio-economic assessment will use a Life Cycle Costing (LCC) approach to quantify and monetize all relevant farm-level costs and changes in input use (e.g. fertilisers, pesticides, fuel, labour) as well as outputs (e.g. yield, returns) associated with the adoption of the new WOSR hybrids. Building on the LCC results, a Cost–Benefit Analysis (CBA) will be conducted to compare aggregated costs and benefits under defined scenarios. Moreover, qualitative social aspects will be considered, and results may be further linked to selected United Nations Sustainable Development Goals (SDGs) to contextualise broader societal implications of trait enhancements. The analyses build on empirical datasets generated within INTEGRA, particularly field trial data and phenotyping data from our project partners. Continuous feedback with other work packages ensures consistency of assumptions and supports iterative refinement of scenarios and indicators.

The first steps of the LCA stages will be defined according to project objectives and progress. The data requirements and time horizon considered in the analysis will support the scenario development, taking into account climatic conditions and genotypic performance in WOSR. The business-as-usual baseline scenario will be used to contrast the prospective scenarios across assessments and supports relevant decision-making.

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Bias and wavelength heritability – a critical view on phenomic selection in rapeseed

Andris J. Finkbeiner[1], Anika Unger[1], Rod Snowdon[1], Benjamin Wittkop[1], Lennard Roscher-Ehrig[1], Sven Weber[1]

Phenomic selection has emerged as a promising yet controversially discussed alternative to genomic prediction (Dallinger et al., 2023; Roscher-Ehrig et al., 2024). Its appeal lies in the relatively low cost of marker acquisition and the increasing availability of phenotypic data. Crops like rapeseed, in which Near Infrared Spectroscopy (NIRS) is routinely applied for assessment of seed quality, can benefit from phenomic selection without any additional costs as the spectral data is already collected routinely in breeding programs.

The prediction accuracy for NIRS based prediction models can vary greatly between crops, sample, and trait (Rincent et al. 2018; Robert et al. 2022a; Zhu et al. 2021). Roscher-Ehrig et al. (2024) compared GS models with PS models, demonstrating that PS has comparable and sometimes higher prediction accuracy than GS models. In contrast, several studies have shown that PS often exhibits lower prediction accuracy than GS, especially for monogenic traits, with certain tissues like wood delivering poor predictions (Brault et al. 2022; Gebreselassie et al. 2017; Rincent et al. 2018). However, the economic superiority of NIRS-BLUP models in comparison to different genomic marker-based models is high: Rincent et al. (2018) estimated an expected selection gain for grain yield in a set breeding program budget in the range from -10% to 222%, depending on the tissue and environment. Interestingly, they also showed that the expected gain with NIRS-BLUP models is significantly lower for less complex traits, such as rust resistance (-10-21%) in wheat.

Despite this potential, several challenges hinder its effective implementation. Among these, the quality of spectral wavelengths is frequently debated as a key factor influencing model performance. Both, Montesinos-López et al. (2017) and Robert et al. (2022) proposed to use only wavelengths with high heritability for NIRS-based phenomic prediction, since they are likely to have greater influence on the predictive ability than wavelengths with lower heritability. Heritability provides information on the degree to which genotypic variance contributes to overall variance and depends on the variance components of a given model (Schmidt et al. 2019). Genotypic variance, in this case, refers to the genotypic variance for each wavelength. Thus, high genotypic variance for each wavelength allows for the selection of cultivars that exhibit genotype-specific reflection patterns, which can be linked to traits by directly referring to trait-dependent molecular patterns (Robert et al. 2022b).

Here we demonstrate that heritability alone does not fully capture the predictive importance of individual wavelengths. Owing to the high degree of collinearity among wavelengths, only a small fraction of approximately 1.1% is necessary for accurate trait prediction. Moreover, PS models often rely on molecular proxies that are correlated with the target trait. In rapeseed, for instance, protein or oil content are highly correlated to seed yield and lead to a potential bias for such traits, which may ultimately reduce the response to selection and undesired selection pressure for correlated traits. In contrast, we found that other traits like flowering time are lacking such molecular correlations and appear to be unaffected by this issue.

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Effects of combined heat and drought on root and shoot development in faba bean *(Vicia faba)*

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Faba bean *(Vicia faba)* is a cold-adapted legume with high yield potential and protein content and quality. However, abiotic stress is particularly harmful for faba bean yield stability during reproductive stages, which is expected to occur more often under current climate change prospects. To allow efficient breeding strategies for more climate-resilient faba bean cultivars, we need to understand the factors influencing yield building under complex stress scenarios. Here, we characterized twelve contrasting spring faba bean accessions in two different facilities: once in a semi-autonomous container facility allowing for realistic root growth and transpiration tracking, and once in mini-rhizotrons for dynamic root architecture parameters. In both facilities, we subjected the plants to both heat and combined heat and drought treatments and measured physiological, architectural and reproductive traits. Separate trials to isolate control from drought scenarios are currently ongoing. The results show that heat response is clearly different from combined heat/drought response, and different accessions show suitable tolerance traits depending on the scenario. For stress in the vegetative stage, combined heat/drought almost completely abolished stomatal conductance, growth and nodulation, while heat alone increased stomatal conductance and was not detrimental to growth and nodulation. Similarly, in the reproductive stage, stomatal conductance decreased under combined stress and increased under heat stress, although heat stressed plants downregulate stomatal conductance later. In contrast to the vegetative stage, chlorophyll content decreased in the reproductive stage, and more quickly under combined stress than under isolated stress. Moreover, the container trial showed that after-stress compensation is an important tolerance strategy in faba bean, specifically so for combined stress.

Partners

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Impact of drought and heat stress on nectar production and pollinator behaviour in faba bean *(Vicia faba* L.*)*

M. Krebs[1], L. Brünjes[1]

Faba bean is cultivated for its protein-rich seeds. The crop relies on bees for high seed set, but bees do not always pollinate the flowers when they visit. Only so-called 'legal' flower visits lead to improved seed set, while nectar robbing does not. The interaction between abiotic stress, flower traits, and pollinator behaviour has barely been studied. Here, we investigate the effects of combined drought and heat stress on nectar traits, as well as on pollinators and their behaviour. In a field experiment covered by a rainout shelter, we grew 24 spring faba bean genotypes, exposing the plants to increased temperature and two water regimes: irrigated and drought-stressed. During the plants' flowering period, we assessed the volume of nectar and the sugar concentration per flower, and conducted observations of the pollinators. Each observed bee was identified at species level, and its specific foraging behaviour (pollination versus nectar robbing) was recorded. Drought stress significantly altered floral rewards, characterised by a decrease in nectar volume and an increase in sugar concentration. Furthermore, drought stress led to a reduction in the number of open flowers and the overall rate at which bees visited the flowers. The percentage of pollinating bees was higher in the irrigated treatment than in the drought-stressed treatment. Our study demonstrates that abiotic stress not only directly limits plant physiology but also indirectly impairs yield potential by reducing floral attractiveness and pollinator activity.

Keywords:
Vicia faba, abiotic stress, plant-pollinator interactions, floral rewards

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Symbiosis-recruited genetic traits for sustainable faba bean production

D. Talwar[1], C. Birkenstock[1], U. Micknass[1], P. Schäfer[1]

Background

The legume crop faba bean *(Vicia faba)* is a rich source of protein for food and feed and is cultivated world-wide. In addition to its high nutritional value, it can be invaluable in sustainable crop rotations leaving surplus nitrogen in the soil demonstrating its high biological N_2 fixation ability. However, the low biotic and abiotic stress resilience of present faba bean cultivars results in low yield stability and production. For these reasons, faba bean grows on only 0.7% of the arable land in Germany (FAOSTAT). The development of more resilient faba bean cultivars is therefore most important. Drought stress is a major constraint in yield stability of faba beans by affecting flower development and reproduction. It can further reduce the size and activity of the nitrogen fixing nodules in grain legumes. In addition to enhancing nutrient supply, beneficial microbes can protect plants against stress. This phenomenon resembles plant systemic immunity in response to a prior stress and is defined as priming, representing a genetically determined plant process that induces higher tolerance against stresses. To access this still untapped beneficial potential of microbes requires the analysis of the interactions of below-ground plant-microbe interactions under stress. Priming can be induced by the endophytic fungus *Serendipita indica* in plants. Upon root colonisation, *S. indica* provides systemic (leaf) and local (root) resistance against diseases and tolerance against abiotic stresses, including drought stress tolerance in a broad range of legumes. As a clearly determined genetic trait, we aim to identify gene candidates that mediate priming-induced protection against drought stress in faba bean.

Methods

Phenotyping of S. indica induced priming will be conducted under control and drought conditions in a panel of 12 extreme cultivars including the reference cultivar Tiffany. Quantification will be done for various vegetative and reproductive traits, photosynthetic efficiency, transpiration, phytohormone levels and fungus colonization. Transcriptomics analysis will be done across for treatments (control, S. indica inoculated, drought and combined) to study differential expression and gene ontology followed by identification of candidate transcription factors using paired motif enrichment tool. Microbiome responses will be assessed using 16S rRNA amplicon sequencing of soil, rhizosphere and endosphere samples.

Expected results

We will investigate transcriptional and microbiome signatures associated with genotype-specific differences in symbiont-mediated drought tolerance.

Conclusion

Key regulators of symbiosis driven drought resilience will be anticipated using phenotypic, transcriptomic and microbiome data. The findings will promote symbiosis based approaches for sustainable faba bean production and will further support marker development and breeding strategies for drought resilient faba bean genotypes.

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DNA-Free CRISPR/Cas9 Genome Editing for Improving Abiotic Stress Tolerance in Faba Bean *(Vicia faba* L.*)*

Amitha Paul, Sarah Schiessl Weidenweber, Babette Knoblauch, Sruthy Maria Augustine

Abiotic stresses such as drought and heat pose major threats to crop productivity and global food security, with climate change further intensifying their impact. Precise genome manipulation is therefore essential for functional gene validation and the development of stress-resilient crops. CRISPR/Cas9 genome editing has emerged as a powerful tool for targeted gene modification, while DNA-free approaches using ribonucleoprotein (RNP) complexes offer distinct advantages by avoiding foreign DNA integration and enabling the generation of plants comparable to those produced through conventional breeding.

In this study, we aim to increase abiotic stress tolerance in faba bean (*Vicia faba* L., cultivar Tiffany) using DNA-free CRISPR/Cas9-mediated genome editing. Two stress-responsive genes, *ACTIN DEPOLYMERIZING FACTOR (ADF)* and *ESKIMO1 (ESK1)*, key regulators of cytoskeletal dynamics and cell wall modification, were targeted. Multiple guide RNAs were designed for each gene, including multiplexed combinations, to improve the mutation efficiency. Preassembled CRISPR/Cas9 RNP complexes were be introduced into faba bean embryos using a novel electric current-mediated delivery method. Regenerated plants were screened for targeted mutations using high-resolution melting analysis (HRMA) and Sanger sequencing, followed by physiological evaluations under controlled abiotic stress conditions. This approach establishes a DNA-free genome editing strategy for improving abiotic stress tolerance in faba bean.

Developmental plasticity and physiological resilience of Faba Bean *(Vicia faba* L.*)* under moderate heat stress

Q. Zhu[1], F. Gahramanli[1], L. Wu[1]

Faba bean *(Vicia faba* L.*)* is a protein-rich legume of paramount agronomic and ecological importance in sustainable cropping systems. However, climate projections for Germany indicate that temperatures of 28–30°C during critical growth phases will become increasingly frequent. High temperatures are known to alter development, reduce cell expansion, affect photosynthesis, and modify nutrient uptake. In legumes, heat stress typically triggers the overproduction of reactive oxygen species (ROS), leading to lipid peroxidation and reduced pollen viability, which often results in significant yield gaps (Janni et al., 2022; Ozga et al., 2017). Furthermore, moderate heat often disrupts the source-sink relationship, where increased respiration rates outpace carbon fixation; yet, some genotypes exhibit remarkable plasticity in maintaining biomass through rapid architectural adjustments (Ferguson et al., 2023). It is therefore critical to understand how this species responds to moderate warming to ensure future food security.

To investigate temperature-induced responses, a greenhouse experiment was conducted with 25 diverse faba bean accessions grown under control (22–25°C) and elevated temperature (28–30°C) conditions. Traits analyzed included plant height, internode length, node and leaf number, shoot and root biomass, chlorophyll content, and photosynthetic activity. We also utilized thermal imaging to monitor leaf temperature and chlorophyll a fluorescence to assess the efficiency of Photosystem II.

The results revealed a significant architectural shift starting at the fourth node, where reduced internode length produced shorter, more compact plants, even as the total node number increased. Surprisingly, despite this reduced shoot elongation, shoot fresh and dry weight, as well as root dry weight, increased under elevated temperatures. Photosynthetic performance was largely maintained, providing the energetic basis for this sustained biomass accumulation.

This reduced stem elongation suggests a temperature-sensitive regulation of cell expansion, potentially mediated by altered gibberellin (GA) biosynthesis or distribution. The ability of these 25 genotypes to shift their morphology without sacrificing biomass indicates a high degree of developmental and physiological plasticity. This compact growth habit may represent an adaptive strategy to minimize transpirational surface area while maximizing structural efficiency.

Our findings demonstrate that faba bean exhibits robust plastic responses to moderate warming. The transition to a more compact architecture while maintaining or even increasing biomass suggests that current germplasm contains significant resilience traits. These results provide a vital baseline for breeding programs aiming to develop climate-smart legumes for Central European agriculture.

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Accelerating Sugar Beet Breeding for Climate Resilience Through Genome Editing and Advanced Aerial Phenotyping

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Sugar beet breeding in Europe faces increasing pressure from climate change, which intensifies drought, heat stress, and extreme weather, threatening yield stability. At the same time, the spread of diseases and pests such as SBR and Stolbur is rising, while regulatory restrictions limit pesticide use. Developing high-performing varieties that combine strong agronomic traits, stress tolerance, and high sugar yield remains a slow and resource-intensive process.

Advanced molecular approaches, including genome editing, offer significant potential to accelerate genetic improvement, provided that technical barriers as well as legal and societal acceptance constraints can be addressed. In parallel, UAV-based high-throughput phenotyping enables rapid, repeatable, and non-destructive assessment of large breeding trials. Multispectral and thermal imaging allow early, objective detection of traits such as biomass development, leaf area index, plant water and nutrient status, and disease infection.

In the BeetAdapt project, Strube integrates genome editing and advanced phenotyping using its breeding material. Agrobacterium-mediated transformation is employed to evaluate the feasibility and efficiency of genome editing using BMC endonucleases in tobacco and sugar beet. Following optimization of genetic transformation protocols for sugar beet, a recalcitrant species, research will target nematode resistance, drought tolerance, and bolting behavior. An X-ray-based high-throughput platform is being evaluated to assess drought tolerance at the seedling stage. UAVs equipped with multiple sensors are used to monitor field plot development and quantify disease pressure, while automated algorithms for sugar beet disease detection are being developed to enable high-accuracy phenotyping.

By integrating these technological advances, BeetAdapt aims to establish a roadmap for incorporating genome editing and advanced phenotyping into accelerated breeding pipelines, thereby strengthening the competitiveness of the German breeding sector and enhancing the resilience of agricultural supply chains.

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Establishing Efficient Particle Bombardment and Genome Editing Tools for Sugar Beet

Sruthy Maria Augustine[1], Rod Snowdon[1]

Sugar beet production faces increasing pressure from climate variability and shifting The Beet-Adapt project aims to substantially reduce the time required to develop sugar beet varieties with improved adaptation to climate change and enhanced stress tolerance. A key challenge in sugar beet research is the crop's high recalcitrance to genetic manipulation, highlighting the need for efficient, reproducible, and genotype-independent transformation and genome-editing systems. Within this framework, Justus Liebig University Giessen (JLU) focuses on the establishment and optimization of particle bombardment–based transformation approaches as a robust alternative to conventional methods.

To standardize particle bombardment–mediated transformation in sugar beet, critical physical and biological parameters—including helium pressure, target distance, particle size, and explant type—are systematically evaluated. Transformation efficiency is assessed using a fluorescent reporter construct, enabling rapid visual identification of successfully transformed tissues. In parallel, the functionality of BMCs and CRISPR/Cas9 genome-editing tools is validated in tobacco through targeted disruption of the phytoene desaturase (PDS) gene, using the albino phenotype as a visual indicator of editing efficiency. Building on these optimizations, BMCs- and CRISPR/Cas9-based genome editing systems are subsequently introduced into sugar beet to target endogenous trait-related genes. Genome-edited events are initially screened using high-resolution melting analysis (HRMA) and further confirmed by Sanger sequencing. In addition, molecular consequences of genome editing are evaluated by quantifying transcript levels of target and associated genes using quantitative real-time PCR (qRT-PCR). Overall, this work establishes a reliable particle bombardment-based transformation and genome-editing platform incorporating both BMCs and CRISPR/Cas9 technologies. This platform provides a critical technological foundation for accelerating the development of climate-resilient sugar beet varieties within the BeetAdapt project.

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NIRS based phenomic prediction of yield traits in sugar beet breeding program

H. Piepho[1], M. Peddu[1], Müller[2], M. Patel[2]

The genetic improvement of sugar beet (Beta vulgaris L.) relies heavily on the extensive evaluation of test crosses across diverse environments to identify stable, high-yielding genotypes. However, the requirement to screen a vast number of hybrids in multi-environment trials (MET) presents a significant bottleneck when budgets are limited. One tool that has gained recent attention is phenomic prediction, where breeders use Near-Infrared Spectroscopy (NIRS) as a high-throughput, cost-effective alternative to genomic markers. This data is routinely generated as part of MET trials at Strube during seed quality analysis and during harvest of sugar beets. Additionally, Strube is generating spectral information using drones during this project. Through this BeetAdapt project, the University of Hohenheim aims to develop and validate phenomic prediction models and methodologies that will allow breeders to utilise the spectral information to optimise resource allocation and improve genetic gain in sugar beet. To this end, we have initiated the first phase of the project where we are implementing and validating phenomic prediction models using MET data from test hybrids grown during the 2024 and 2025 growing seasons, respectively. We applied a two-stage modelling approach. In Stage 1, phenotypic Best Linear Unbiased Estimators (BLUE) were estimated for corrected sugar yield (CSY), corrected root yield (CRY), and sugar content (SC) using a linear mixed model accounting for environmental and design effects. In Stage 2, these BLUEs served as response variables in a Ridge Regression Best Linear Unbiased Estimators (BLUP) using standardized NIRS data as predictors. Predictive ability was assessed using fifty replications of a five-fold cross-validation scheme. Performance was evaluated using Pearson's correlation, Root Mean Square Error (RMSE), and the Mean Squared Error of Predicted Differences (MSEPD) to assess ranking accuracy. Early results indicated that random regression significantly outperformed BLUP using a relationship matrix derived from spectra and showed good prediction ability that is trait-dependent, especially in cases like ours where the number of genotypes is higher than the number of spectral wavelengths. These findings suggest that NIRS-based phenomic prediction can successfully predict total genotypic value, enabling breeders to discard inferior germplasm prior to expensive field testing. We are expanding the model to include spectra-by-environment interactions, information from drones and evaluating the performance of models in different breeding scenarios. This approach will facilitate more efficient allocation of resources, allowing for larger populations to be screened or more resources to be redirected toward high-potential test crosses.

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Development of new UAV methodology for autonomous scoring

Jonas Bömer[1], Anne-Katrin Mahlein[1], Stefan Paulus[1]

Sugar beet production faces increasing pressure from climate variability and shifting pest and disease dynamics, while the availability of established chemical plant protection is being reduced. In parallel, breeding programs need faster and more objective field measurements to select resilient genotypes. Today, many key traits are still assessed by manual visual scoring, which is labor intensive and can vary between observers. These factors together create a need for scalable, objective and timely quantification of plant stresses to support selection decisions in breeding nurseries and field trials.

Therefore, the project *BeetAdapt* aims to develop an autonomous scoring methodology that links optical sensing with artificial intelligence running directly on sensor hardware, so that meaningful outputs are produced during data acquisition rather than after extensive offline processing. Providing these instant classifications can reduce the need for data handling, improve consistency across sites and seasons, and enable faster feedback during the field season.

The targeted use case is the detection and scoring of the disease “syndrome basses richesses” (SBR), which is monitored in field trials and requires timely, manual assessment. In addition, the methodology is intended to be extensible, and premature flowering is considered as a potential secondary application to test whether the sensing and embedded workflow can be applied to another distinct, visually assessable trait. Together, these use cases cover both abiotic and biotic stress and provide a practical pathway to evaluate the robustness and transferability of autonomous field scoring.

The project schedule involves a staged pipeline from point spectroscopy to aerial imaging. First, stress-related optical signatures of SBR are recorded using a manually operated, handheld spectrometer. These measurements establish a robust training and validation basis for sensor-adapted models. Secondly, the models are adapted to the restrictions of embedded on-device computing and transferred to an EdgeAI spectrometer to create an integrated sensing and detection unit. The quality of the developed sensor is evaluated during an additional period of field data collection.

Finally, this concept is extended from one-dimensional handheld spectroscopy to two-dimensional imaging suitable for deployment on uncrewed aerial vehicles. Therefore, a camera-based sensor, combined with embedded computing, is developed and used to integrate models trained on aerial color imagery for autonomous scoring of experimental plots in SBR field trials. Finally, the developed imaging system is validated against conventional offline workflows and expert scoring. This shift from collecting only raw images to producing on-site outputs in the field is intended to streamline data handling and enable immediate, standardized stress assessments across plots.

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Advancing Sugar Beet Genome Editing with Laser-Assisted Transformation and Novel Nucleases

N. Raven[1], S. Di Fiore[1], A. Schiermeyer[1]

Sugar beet is a major crop in Germany and across the EU, yet it remains highly recalcitrant to genetic transformation and genome editing using established methods. The BeetAdapt project aims to accelerate the development of climate-adapted, stress-tolerant sugar beet varieties by establishing an integrated toolbox of advanced breeding and genome editing technologies. A central objective is the development of efficient, robust, and reproducible transformation and genome-editing pipelines that overcome current technological limitations. Within this framework, Fraunhofer IME exploits an innovative DNA-free delivery platform and evaluates novel genome editing nucleases to enable precise, genotype-independent modification of sugar beet.

A key technological innovation is laser-induced shock wave transformation (LIST), which uses laser-generated shock waves to transiently loosen the cell wall and plasma membrane, allowing delivery of genome editing reagents into intact cells and tissues. LIST overcomes the need for protoplast preparation is efficient and gentle in transfection, thereby preserving regeneration potential of the transfected cells. The laser-assisted transformation method requires minimal preparation, is applicable across tissues and genotypes and compatible with all types of SSNs. These properties make LIST particularly attractive for application in recalcitrant crops such as sugar beet.

Genome editing is performed via delivery of preassembled ribonucleoprotein complexes (RNPs) composed of a nuclease and guide RNA, enabling DNA-free editing and recovery of transgene-free plants. In addition to CRISPR/Cas9 RNPs, we investigate CRISPR-BMC endonucleases, a proprietary metagenomics-derived nuclease family (BRAIN Biotech AG) that has demonstrated high precision and low off-target activity in microbial systems but has not yet been systematically validated in plants.

In the first project phase, a LIST protocol for sugar beet is established using DsRed fluorescent protein and labeled RNPs to define delivery conditions and rapidly identify transfected tissue. BMC RNPs are produced, purified, and tested for in vitro activity, followed by functional validation and benchmarking against Cas9 RNPs in tobacco. LIST-mediated RNP delivery is then applied to targeted knockout of endogenous visual marker genes in sugar beet. Editing events are confirmed by high-resolution melt analysis, fragment length analysis, and sequencing.

Together, this work will establish a reliable, DNA-free transformation and genome editing platform for sugar beet to accelerate crop improvement.

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Untargeted metabolite profiling

C. Schulze Gronover[1], B. Müller[1], N. van Deenen[1]

Buckwheat *(Fagopyrum esculentum)*, a pseudocereal, was once a staple food in Germany but was replaced by cereals with higher yields several decades ago. It contains various phytochemicals not only in its grains but also in its leftover biomass, including leaves, stems, and husks. Buckwheat is particularly notable for its high rutin content, which is valued by the pharmaceutical industry for its beneficial health effects. Today, buckwheat is becoming more popular again as a gluten-free and nutritious alternative to wheat, though it currently needs to be imported. To revive buckwheat cultivation in Germany, breeding efforts for high-yielding varieties suited to present climate conditions are necessary. In accordance with the National Bioeconomy strategy to enhance the use of agricultural by-products, BIMOTEC is assessing buckwheat's potential as a versatile crop. Beyond using the grains for food, the project explores extracting valuable phytochemicals from leaves and husks and lignocellulose from stems in the residual biomass. Through an interdisciplinary approach, the BIMOTEC partner address various aspects to advance buckwheat breeding and production in Germany.

Given the renewed interest in buckwheat as a valuable, multi-purpose crop in Germany, Fraunhofer IME plays a crucial role by conducting comprehensive GC-MS and LC-MS analyses of leaf material from fifty buckwheat cultivars originating from 20 different countries. This in-depth metabolite profiling and the search for valuable secondary metabolites are essential steps toward identifying compounds with significant industrial potential. By uncovering and utilizing these metabolites, the project aims to enhance the economic viability of buckwheat cultivation, supporting efforts to re-establish this crop in Germany and fully exploit its potential in line with bioeconomy strategies.

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Establishment and utilization of genome editing to render common buckwheat a crop for modern agriculture and molecular farming

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Buckwheat belongs to the genus *Fagopyrum* of the *Polygonaceae* family. The two main cultivated species are common buckwheat *(Fagopyrum esculentum)* and tartary buckwheat *(Fagopyrum tataricum)*. Buckwheat is a globally grown pseudo-cereal that was domesticated in southwest China. Most of the pseudo-cereals are dicotyledonous plants that are different from cereals in plant architecture and seed/fruit structure. Although buckwheat has the name “wheat” it is not related to wheat, but its seeds can be used for gluten-free and nutrient-rich flour production. Buckwheat is currently used as catch crop and as bee pasture plant in Germany.

In the BIMOTEC project, buckwheat will be optimized for modern agriculture via genome editing. For this, we are facing a critical need for robust methods of adventitious shoot formation *in vitro* and genetic transformation. Here we present first results on surface disinfection of the seeds and the formation of hypocotyl and cotyledon-derived callus giving rise to multiple shoots. We are currently using this regeneration principle to establish the *Agrobacterium*-mediated transformation of buckwheat.

In the further course of the project, it is envisioned to introduce semi-dwarfism into buckwheat, as this trait has revolutionized agriculture by improving the yields of several important crops. Shorter buckwheat varieties will have a higher harvest index and thus a higher yield. Additional putative target genes are currently being identified by the project partners. These genes relevant to the goals of the BIMOTEC project, such as drought tolerance and increased content of nutritionally and pharmacologically valuable metabolites, are also planned to be modified through genome editing.

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Accounting for Spatial Heterogeneity in Field Phenotyping of Cabbage Stem Flea Beetle Damage as a Basis for GWAS

L. Roscher-Ehrig[1], B. Wittkop[1], C. Obermeier[1]

One of the major aims of Res4StRes is to identify genetic resources for resistance breeding against cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*) in diverse Brassica germplasm. In the first phase of the project, seeds of approximately 280 diverse genotypes, including old line varieties, fodder rape, exotic accessions, and resynthesized lines, were multiplied and distributed to project partners. Eight field experiments across different locations within Germany and three bioassays were initiated.

At Justus Liebig University Giessen, four field experiments were established at three locations (Rauischholzhausen, Weilburger Grenze, and Groß-Gerau) focusing on insect resistance and sulfur use efficiency. Each experiment comprised 300-400 plots. To quantify feeding damage by adult CSFB, around 900 barcoded digital images per location were collected at two time-points and visually evaluated. The phenotyping and analysis pipeline was iteratively refined in collaboration with the project partners from Georg August University Göttingen to ensure standardized scoring and traceability.

Preliminary analyses of leaf damage revealed pronounced spatial heterogeneity within fields. Patterns of infestation indicated strong local effects, most likely driven by immigration of beetles from surrounding fields and flowering strips. To account for these gradients, replicated control genotypes distributed across the field were used to model and adjust for spatial trends to ensure comparability among genotypes. After correction, ranking of genotypes across the three locations revealed a subset of lines from different genetic subgroups that consistently exhibited comparatively low levels of infestation. These genotypes could represent promising candidates for further validation and potential resistance breeding.

The experiments will be repeated at the same locations in the coming years to capture interannual variation and to strengthen the multi-environment dataset. Refined phenotyping approaches and spatial adjustment strategies established in this year provide a basis for the evaluation of these future trials. This will allow to generate a robust dataset as a reliable basis for subsequent GWAS aimed at identifying loci associated with CSFB resistance.

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Video tracking – A high-throughput visual & camera-based controlled bioassay in laboratory for the green peach aphid Myzus persicae

M. Kern[1], T. Will[1]

Rapeseed *(Brassica napus)* is an important, globally grown oil plant, used for food and technical applications. The ban of neonicotinoids for seed treatment that were able to control insect pests in the past increases the risk of insect damage. The green peach aphid *Myzus persicae* causes economically significant damage to rapeseed crops, among other things by transmitting the turnip yellows virus (TuYV). The problem is exacerbated by the fact that aphids benefit from climate change and, as a result of milder winters and longer warm periods in autumn, are active in the field for longer periods. Our aim is to identify and characterize resistances against *M. persicae* by applying innovative phenotyping and genomic tools, metabolomic pathway analysis and bioinformatic approaches to systematically exploit existing and novel biodiversity for integrative rapeseed crop improvement. For greenhouse phenotyping of plant-insect interactions in high-throughput bioassays, we are using a camera-based video tracking method. The initial screening, based on activity tracking on leaf discs, of 180 rapeseed genotypes has been initiated. As a basis for this, *M. persicae* of various origins were characterized in terms of their feeding behavior on *B. napus*, which was measured using the Electrical Penetration Graph (EPG) technique. The findings were incorporated into the selection of biotypes for video tracking and were used to interpret the results of the video tracking data.

Partners

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Novel resources for resistance breeding against insect pests and heat stress under low cropping intensities in oilseed rape – Subproject B

A. Ebert[1], T. Meiners[1], C. Böttcher[1], N. Austel[1]

Aim

Insect damage, heat stress and sulphur deficiency are current threats to rapeseed *(Brassica napus)* production in Germany. In addition to that, rapeseed is one of our most inbred crop species harboring little genetic and phenotypic diversity within the primary germplasm pool. In this project we aim within a consortium of entomologists, metabolomics experts, plant breeding researchers, bioinformaticians and commercial breeding companies to identify and characterise biotic and abiotic resistances for integrative rapeseed crop improvement. This will be achieved by developing and applying innovative phenotyping, genomic and metabolomic pathway analyses and by using bioinformatic approaches to systematically exploit existing and novel biodiversity in oilseed rape and other Brassicaceae species including hybrids thereof, where the JKI-ÖPV is involved in the production of hybrid plant germplasm sets.

Resistance phenotyping

The JKI-ÖPV is phenotyping the feeding damage by pollen beetles *(Brassicogethes aeneus)* in field trials located at the breeding companies and Justus Liebig University (JLU) and; JKI-ÖPV will screen for plant resistance against the cabbage root fly *(Delia radicum)* under controlled conditions. For this purpose, a constant rearing of the cabbage root fly has been established at JKI-ÖPV. Feeding bioassays are being conducted on intact plants from the project's germplasm sets. To identify susceptible and resistant plant accessions against *D. radicum*, a high-throughput phenotyping method will be developed by JKI-ÖPV using video tracking of insect behaviour in-vitro.

Metabolomics phenotyping

JKI-ÖPV will perform analyses of semi-polar secondary plant metabolites using targeted and non-targeted analytical approaches. The obtained metabolic fingerprints will be correlated with observed insect performance parameters of the cabbage stem flea beetle, the green peach aphid and the cabbage root fly obtained by Georg-August-Universität, JKI-RS and JKI-ÖPV. In cooperation with JLU a new near-infrared spectroscopy (NIRS) method for glucosinolates in non-seed organs will be established and validated. To be able to screen a high number of different genotypes, a high-throughput glucosinolate extraction method using 96-well plates and a vacuum manifold is being employed (Beran et al., 2014). For testing the applicability of NIRS to field-harvested non-seed organs, the impact of different harvesting and processing methods on the glucosinolate profile are being investigated. JKI-ÖPV is performing quantitative glucosinolate analysis of different tissues of B. napus, that are the feeding target of the studied pest insects.

Conclusion

We expect to identify climate-robust resistances against the targeted insect pest species together with the project partners. Metabolomic pathway analysis will enable us to transfer knowledge also from wild species to B. napus to provide metabolome bio marker and molecular marker assays for use in marker-assisted introgression breeding of oilseed rape by the breeding industry.

Partners

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Phenotyping cabbage stem flea beetle resistance in oilseed rape using field and controlled bioassays

C. Posada-Vergara[1], M. Rostás[1]

Damage to oilseed rape *(Brassica napus)* caused by the cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*) has intensified in Germany following the loss of effective chemical control options, including widespread pyrethroid resistance and the ban of neonicotinoid seed treatments. As a result, yield losses have increased and oilseed rape cultivation has been discontinued in some regions. Effective genetic resistance in B. napus is currently limited, and robust phenotyping approaches are required to identify resistance traits suitable for breeding.

To identify resistance traits against CSFB, we focus on three complementary approaches: (i) assessment of adult feeding damage in field trials, (ii) quantification of larval infestation levels in field-grown plants, and (iii) controlled feeding bioassays with adult beetles.

In the first project year, adult feeding damage was documented in the Res4StRes field trials using a standardized image-based protocol applied across consortium locations. Standardized plot images provided by the breeders at early growth stages (BBCH 10–11 and BBCH 13–14) are currently being evaluated. Damage scoring is based on manual visual assessment, and different evaluation approaches are being compared.

To assess resistance expressed through reduced larval infestation, we developed a modified Berlese-based method to quantify CSFB larvae in plants sampled from Res4StRes field trials. Larval numbers are used as an indicator of resistance, integrating effects on oviposition, larval penetration, and early development. Field sampling is scheduled prior to the meeting.

In parallel, an established feeding bioassay is applied and further optimized under controlled conditions to assess plant resistance to feeding by adult CSFB. A set of 100 genotypes from the project material was selected as a subset, prioritizing lines with contrasting responses in previous datasets for adult feeding damage and/or larval infestation. Screening is ongoing, and first comparative results are becoming available.

In addition, protocols for targeted metabolite analysis are currently being optimized to support the integration of phenotypic and metabolic data in subsequent project phases

Partners
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First genomes in a common platform

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Insect herbivory, heat stress, and sulfur deficiency are increasing threats to oilseed rape (Brassica napus) production under climate change. The Res4StRes project aims to identify biotic and abiotic resistance traits by combining phenotyping, genomics, metabolomics, and bioinformatics. Diverse Brassicaceae breeding populations are analyzed to identify resistance-associated metabolites and candidate genomic loci. Resistant genotypes are further evaluated under heat and sulfur stress to support climate-resilient breeding strategies.

Due to the interdisciplinary approach in this project, many different types of data will be generated. A particular challenge is the complexity of the data, which will represent very different aspects ranging from plant phenotyping, metabolomic and genetic data to insect infestation assessments to e.g. sulfur and abiotic stress status.

To address this challenge and ensure sustainable research data management, a dedicated web-based platform has been established and is available at res4stres.de. This platform serves a dual function: it publicly disseminates the project's progress and goals while simultaneously providing a secure environment for data exchange among collaboration partners. All generated data will be shared and uploaded via the website and backend annotated and uploaded into DataPLANT ARC containers to archive and manage project data in compliance to the FAIR (Findable, Accessible, Interoperable, Reusable) principles.

In addition, we present novel data on whole-genome sequencing, three Brassica napus genotypes were chosen based on preliminary trials that indicated resistance phenotypes. High-quality reference genomes were generated using long-read sequencing with Oxford Nanopore Technologies (ONT) only. Due to new and improved assembly approaches, nanopore data alone allowed the assembly of three chromosome-scale genomes.

Partners

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References

https://res4stres.de/

Harnessing Novel Genetic Resources for Introgression Breeding of Insect Resistance in Oilseed Rape (Brassica napus) (Res4StRes – Subproject F)

A.S. Mason[1], A. Kaur[1]

Background

Rapeseed *(Brassica napus* L.*)* suffers major yield losses from insect pests such as Cabbage Stem Flea Beetle *(Psylliodes chrysocephala)*, Cabbage Root Fly *(Delia radicum)*, Green Peach Aphid *(Myzus persicae)*, and Cabbage Seedpod Weevil *(Ceutorhynchus picitarsis)*. In Europe, insect pest damage causes roughly 15% annual yield reduction in oilseed rape: insecticides played a major role in controlling this damage until the recent ban on neonicotinoid insecticides further exacerbated pest pressure (Dewar 2017; Milovac *et al* 2017). Modern rapeseed cultivars generally show little insect resistance: to date, no major resistance has been identified within the primary rapeseed germplasm pool. Consequently, the identification and deployment of novel resistance sources may be critical in improving insect resistance in rapeseed. Wild Brassicaceae relatives like *Sinapis alba, Crambe hispanica, Iberis amara, B. fruticulosa*, and *Eruca vesicaria* are an under-exploited reservoir of insect resistance traits. However, introgression breeding to transfer insect resistance traits into *B. napus* is hindered by challenges such as barriers to wide hybridization, limited recombination between *B. napus* and alien chromosomes, and associated linkage drag (Hervé 2018).

Objectives

Firstly, backcrossed populations of intergeneric hybrids between *B. napus* and *Sinapis alba* and *Eruca vesicaria* will be phenotyped for resistance to the target insect pests and further genotyped using molecular markers to identify the chromosomes or chromosomal segments associated with resistance loci. These marker-based analyses will be further integrated with cytogenetic tools like genomic and fluorescence in situ hybridization (GISH/FISH) to validate the observed alien introgressions and correlate them with the resistance phenotypes. The selected individuals will be then treated with gamma irradiation in order to reduce the linkage drag associated with these alien introgressions and to promote recombination between B. napus and alien chromosomes. The recombinant chromosomes will be identified using both molecular and cytogenetic approaches, followed by backcrossing to *B. napus* to recover genetically stable lines.

Ongoing work

This year (2026), we will plant spring-type germplasm sets/material in field trials in spring and record insect damage on young plants. Subsequently, we will focus on performing molecular and cytogenetic analyses to investigate the resistance traits in this material. A similar trial will be planned in the autumn of 2026 to investigate resistance in this material against Cabbage Stem Flea Beetle *(Psylliodes chrysocephala)*. The expected outcomes of this study will be the identification of alien chromosome(s) conferring insect resistance. Subsequently, we will aim to further develop diagnostic molecular markers for the resistance introgression and will work towards producing stable introgression lines of *B. napus* exhibiting robust insect resistance.

Partners

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Phenotyping for pest insect resistance in multiple field environments

S. Abel[1], F. Breuer[2], S. Möller-Haferkorn[3], M. Rakoski[5], S. Rietz[6]

Insect damage, heat stress and sulfur deficiency are current threats to rapeseed production in Germany. In the Res4StRes project we aim within a consortium of entomologists, plant breeding researchers and commercial breeding companies to identify and characterise biotic and abiotic resistances by developing and applying innovative phenotyping, genomic, metabolomic pathway analysis and bioinformatic approaches to systematically exploit existing and novel biodiversity for integrative rapeseed crop improvement.

In the first phase of the project partners from the commercial breeding sector conduct multiple field trials across Germany with the Res4Stres diversity set of Brassica napus germplasm. At different sulfur fertilization regimes, plant development and pest infestation is scored throughout the cropping season. Among others, adult feeding of cabbage stem flea beetle and later of their larvae are of particular interest. The aim is to identify genotypes of superior tolerance to pest feeding and provide data and plant material for genetic and metabolic analysis inside the project consortium.

Partners

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[2] KWS SAAT SE & Co. KGaA | Einbeck, Germany
[3] Deutsche Saatveredelung AG | Lippstadt, Germany
[4] Gemeinschaft zur Förderung von Pflanzeninnovation e.V.
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Genome-to-Genome inference of GxGxE interactions for climate-resilient pathogen resistance in barley

A. Tellier[1], A. Boukherissa[1], P. Wolper[1]

Breeding for durable disease resistance in barley is complicated by genotype-by-genotype-by-environment (GxGxE) interactions. The outcome of host-pathogen encounters depends on the genetic composition of both organisms and local climatic conditions. Classical GWAS mapping approaches treat pathogen diversity and environmental variation as noise rather than signal, limiting our ability to identify resistance loci that remain effective across pathogen populations and changing environments.

The BarleyCOPA project addresses this gap by developing and applying the first Genome-to-Genome (GtoG) association framework for crop breeding. We focus on spring barley and two of its most damaging fungal pathogens in Germany: *Fusarium graminearum*, the causal agent of Fusarium Head Blight (FHB), and *Ramularia collo-cygni*, responsible for Ramularia Leaf Spot (RLS). Both diseases have increased in prevalence and severity since the 1980s, and their management currently relies on chemical crop protection, with consequences for soil biodiversity and sustainability.

Our GtoG framework consists of three integrated modules (Fig. 1). The first module uses a forward-in-time population genomics simulation to model the processes that shape pathogen genetic diversity across field locations. This produces prior distributions for pathogen allele frequencies and population structure. Module 2 fits a Susceptible-Exposed-Infected-Removed (SEIR) epidemiological model to field disease progression data, providing estimates of disease encounter rates for each barley variety and field. Before the inference step, we contribute to barley genome assembly and annotation by processing long-read and short-read barley sequencing data to build high-quality assemblies and performing pan-genome and CNV analyses in collaboration with Helmholtz PGSB. Module 3 then combines all these inputs in an Approximate Bayesian Computation (ABC) scheme that tests all pairwise barley SNP/CNV x pathogen SNP/CNV associations against a set of interaction matrices (Gene-for-Gene, Matching-Allele, host resistance, pathogen virulence, neutral) (Märkle et al., 2024). Significant GxG associations are identified using Bayes factors, and GxGxE effects are detected as shifts in interaction matrices across field environments.

The experimental design involves 200 spring barley lines grown across five field locations in Germany over four years. Over 1,000 isolates each of F. graminearum and R. collo-cygni are sampled and sequenced from three field trial locations over two years.

We are actively advancing multiple aspects of this research. We genotyped 96 barley cultivars using the 15K SNP array. Population structure analysis and diversity metrics were computed, and Core Hunter consensus optimization was applied to identify the most divergent lines. The final selection of cultivars for long-read sequencing was guided by both maximizing genetic diversity and our evolutionary questions regarding host-pathogen coevolution. An additional 76 cultivars are currently being processed for SNP array genotyping. In parallel, we are developing a co-infection epidemiological model and validating it using an existing *wheat-Zymoseptoria tritici* dataset. We are also extending the GtoG ABC framework to handle diploid host data and to allow dominance effects in the interaction matrices. Furthermore, we are exploring the utility of ancestral recombination graphs (ARGs) of hosts and pathogens and their joint signatures under coevolutionary processes, with the hope of using the ARG as an efficient and informative encoding of genomic ancestry that can improve existing genome-to-genome inference methods.

Partners

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Towards Fusarium spec. pangenomics for understanding barley quantitative resistance to Fusarium Head Blight

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Phenotyping FHB in the barley pan genome panel

A part of the BarleyCOPA consortium, we aim at understanding of FHB pathogenesis in barley. FHB often occurs with high toxin loads, even without visible symptoms. Grain from a pan genome panel from diverse field sites is analysed on a yearly basis. For each genotype, 100 kernels are milled for DNA extraction and qPCR targeting *Fusarium genomic* DNA, normalized to barley DNA as a proxy for disease severity and toxin contamination (Hoheneder et al. 2022). Across four years, we will define barley genotypes with robust quantitative resistance (QR) or susceptibility for breeders and for further selection. We optimized methodology for DNA extraction from starch-rich samples and qPCR and semi-automatized processes for high throughput. Data are going to be used for GWAS on barley pan-genome data for the identification or FHB resistance- or susceptibility-associated loci.

Isolation of and pan-genomics of *Fusarium strains*

From each barley sample, we found 20-60% of kernels to contain *Fusarium species* contamination. From 100 kernels per genotype, fungi growing on PDA are regrown, single spore isolated, and confirmed for species level via diagnostic PCR. At least one isolate per host genotype and field site (enabling subsequent GxGxE analysis with TUM-PG (Freising, AG Tellier) will be collected over years 1 to 3 (~1,000 isolates). Genomic DNA from up to 1,000 isolates will be isolated and Illumina-sequenced. Regarding fungal species, for which we lack a reference genome from mid European isolates, we will perform long read sequencing and assemble full genomes as new references with H-PGSB (Neuherberg, AG Spannagl). Single nucleotide polymorphisms, copy-number and presence-absence variation including effector and chemotype genes, will be jointly analysed with CAU PP (Kiel, AG Stam) for population genetics. We have optimized the protocols for fungal isolation from kernel material, isolation and identification of Fusarium species isolates. This characterized a species complex of *F. graminearum, F. culmorum, F. avenaceum, F. tricinctum, F. poae, F. langsethiae, and F. sporotrichioides* with a lack of *F. graminearum dominance* . Data will create a Fusarium-genus pan-genome for better understanding diverse fungal infection strategies.

Confirmation of host QR and fungal aggressiveness

Greenhouse inoculations will validate field observed QR and test GxG interactions in controlled environments. Selected barley and fungal genotypes will be cross tested. Disease will be quantified by head symptoms and qPCR. Results will support controlled environment studies by TUM-ENI (Freising, AG Steidele) and be exploited for studying polymorphic fungal gene expression. Strains differing in aggressiveness will be used for time course RT qPCR on diverse barley genotypes. Candidate loci from GxG analyses and chemotype specific genes (e.g., *tri5*, DON/NIV related genes) will be profiled. Corresponding toxins will be quantified by LC MS/MS. This will reveal fungal gene expression patterns linked to pathogen aggressiveness and chemotypes.

Partners

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Transcriptional Regulation of Barley-Fusarium-Environment Interactions

J. Yang[1], C. E. Steidele[1]

Plants in natural environments are constantly exposed to diverse abiotic and biotic stresses that influence growth, physiology, and defense capacity. Abiotic factors such as drought, extreme temperatures, and elevated ozone or CO_2 interact synergistically, antagonistically, or neutrally through complex regulatory networks, thereby altering plant susceptibility to pathogens [1]. Fusarium head blight (FHB), caused by several *Fusarium species*, remains one of the most destructive cereal diseases, reducing yield and grain quality and contaminating grain with the mycotoxin deoxynivalenol (DON) [2]. While individual stress responses in cereals have been widely studied, far less is known about how combined or sequential abiotic stresses modulate FHB development—an increasingly important question under progressing climate change. Many known FHB resistance QTLs exhibit strong environmental dependency [3], further emphasizing the need to understand stress-pathogen interactions.

Previous work has shown that pre infection drought stress can reduce FHB susceptibility in several barley varieties, whereas simultaneous drought and infection can enhance disease severity [4,5]. Building on these findings, the present study investigates how extreme temperature (35 °C), elevated ozone (100 ppb), increased CO_2 (1000 ppm), and their combination affect FHB progression in barley. Two varieties with contrasting resistance—'Barke' (relatively resistant) and 'Palmella Blue' (susceptible)—are assessed using a standardized spray inoculation protocol with *F. culmorum*.

Disease progression will be monitored visually and quantified using the relative area under the disease progression curve (rAUDPC). Fungal biomass will be measured by qPCR to provide a sensitive estimate of infection severity. Physiological parameters, such as chlorophyll content (SPAD) and stomatal conductance, will be recorded to link stress induced physiological changes to observed disease phenotypes. To determine whether the environmental conditions directly impact fungal fitness, we additionally analyze fungal growth in vitro under identical stress scenarios.

To identify transcription factors (TFs) and regulatory modules associated with barley resistance under combined stresses, we have established a transcriptional regulatory network pipeline [6] integrating differential expression analysis, weighted gene co expression network analysis (WGCNA) [7], and gene regulatory network inference via GENIE3 [8]. Predicted TF–target interactions will be validated in vivo using promoter activation assays.

By elucidating how climate relevant stresses influence the barley–Fusarium interaction at physiological, pathogenic, and regulatory levels, this work aims to guide future strategies for breeding barley varieties with improved resilience under shifting environmental conditions.

Partners

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WP4 – Pathogen population genomics analyses

R. Stam[1], T. Dumartinet[1]

Crop resistance and epidemic severity are driven by the interaction between host and pathogen genotypes, with the outcome being strongly modulated by climatic and environmental conditions. Deciphering Genotype × Genotype × Environment (G×G×E) interactions is crucial for predicting and ensuring the long-term durability of disease resistance in crops. Barley is one of the most widely grown cereal crops in Europe and its production is affected by various constraints and diseases, such as Fusarium Head Blight (FHB) and Ramularia Leaf Spot (RLS). Both diseases have become increasingly widespread and damaging since the 1980s due to changing climatic conditions. Developing crop varieties with durable resistance is essential for sustainable modern agriculture, yet achieving this remains a major challenge due to rapidly evolving pathogens and increasingly variable environmental conditions. The BarleyCOPA project aims to 1) develop and implement the first Genome-to-Genome (GtoG) framework specifically tailored for plant breeding and 2) to identify novel spring barley varieties that exhibit robust resistance to G×G×E interactions, against FHB and RLS. A total of 200 barley lines, including 20 modern reference lines, 50 wild barley and landrace lines and 130 modern breeding lines, will be grown over three years (2026-2028), in five different experimental sites (four in Bavaria and one in Schleswig-Holstein). The integration of phenotypic, genomic, and climatic data will enable project partners to construct a co-pangenome for barley and two of its major pathogens and to predict the future outcome of epidemics by deciphering specific G×G×E interactions.

The main objective of WP4 is to investigate the population genomics and dynamics of Ramularia *collo-cygni* (Rcc) and *Fusarium graminearum* (Fg). For each experimental site, we will evaluate the severity of Ramularia leaf spot (RLS) and Fusarium head blight (FHB) annually for each variety using visual disease scoring. Leaf and seed samples will be subjected to high-throughput DNA extraction and quantitative PCR to quantify Rcc and Fg across varieties and sites. Single-spore isolates of Rcc will be collected from symptomatic leaves across three field plots over two consecutive years. Genomic DNA will be extracted from approximately 1,200 isolates, followed by whole-genome sequencing to generate a single nucleotide polymorphism (SNP) dataset. Equivalent analyses will be conducted by our partners for Fg. Population genetic statistics (e.g. nucleotide diversity, Tajima's D) will be estimated, and population structure will be characterized to investigate spatial and temporal patterns of genetic variation in both pathogens. We will employ simulation approaches to model pathogen migration and evolution, thereby informing projections of future Rcc and Fg risk.

We developed and optimized protocols in a pilot field trial conducted in 2025 across multiple sites in Bavaria and Schleswig-Holstein before the full-scale experiment planned for 2026. Samples collected in 2025 enabled the implementation and optimization of disease scoring methods, high-throughput DNA extraction and qPCR assays, and single-spore isolation of Rcc.

Partners

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Integrative mapping of priming-associated disease responses in a barley diversity panel

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Immune priming in cereals is expected to rely on small-effect loci and context-dependent regulatory mechanisms rather than major resistance genes. Using the PrimedPlant barley diversity panel, we combined linear mixed-model GWAS, machine-learning-based association analyses, and RNA-seq evidence across four independent disease phenotypes. Across methods and datasets, individual association signals were weak, consistent with a quantitative genetic architecture. However, convergence analysis highlighted a limited set of candidate genes with plausible roles in immunity regulation, including an importin on chromosome 1H and two adjacent NAC-domain transcription factors on 2H, particularly in leaf rust responses. While most signals were phenotype-specific, partial overlap of candidate genes across diseases was observed, indicating shared regulatory components underlying priming-related responses. Together, these results show that integrative, multi-evidence approaches can extract biologically meaningful candidates from quantitatively controlled priming traits and provide a robust basis for targeted functional validation and breeding-oriented prioritisation.

Partners

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Bacterial quorum sensing molecule functions as postbiotics but the effects are dependent on substrate

M. Cambeis[1], A. Shrestha[1], M. Grimm[1], A. Schikora[1], O. Moshynets[2]

The use of postbiotics, defined as metabolites produced by microorganisms, represents a promising approach in sustainable agriculture. Their application is often more reliable than the use of living microorganisms.

In this study, we investigated the impact of the *N*-acyl homoserine lactone (AHL), N-hexanoyl-L-homoserine lactone (C6-HSL) on two spring barley genotypes, Golden Promise and BCC436. Both genotypes were already shown to respond to AHL treatment. We assessed plant performance, susceptibility to *Blumeria graminis* f. sp. *hordei (Bgh)*, and treatment-induced changes in the bacterial community of the rhizosphere.

Our results indicated that barley responses to C6-HSL are genotype-dependent and further modulated by the microbiome. In addition, the root-specific transcription factor MYB72 seems to be involved in the response, triggering induced systemic resistance (ISR) to *Bgh*. Although AHL treatment induced shift in the bacterial community, no individual taxa were identified as specifically responsive to C6-HSL treatment. This suggests a general but subtle restructuring of the microbial community.

Overall, C6-HSL application induced physiological changes in barley and triggered a top-down effect on the rhizosphere microbial community.

Partners

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Field Application of Beneficial Rhizobacteria for Enhanced Resistance and In Vitro Conidiation for Controlled Infection of Barley

S. Raetz[1], M. Cambeis[1], A. Schikora[1], Y. Becker[1]

Ramularia leaf spot (RLS) disease is increasingly impacting barley farming in Germany. The causal agent of this disease is the hemibiotrophic fungus *Ramularia collo-cygni* (Rcc). Rcc remains inside the plant without causing symptoms throughout the growing season and only becomes noticeable late in the season with significant sporulation and visible symptoms. Addressing RLS in the field poses several challenges, such as a difficult diagnosis, the absence of resistant barley varieties, and the ongoing reduction in the effectiveness of commonly used fungicide classes.

The PrimedPlant-3 project investigates the application of beneficial rhizobacteria (*Bacillus velezensis* and *Bacillus pumilus*) to enhance the broad-spectrum resistance of barley against pathogens by conferring induce systemic resistance (ISR). To evaluate the effect of bacterial seed inoculation on RLS disease development, multi-site field trials were conducted in Germany over two consecutive years. Eight barley lines exhibiting high genetic variability were included. During the typical outbreak period, coinciding with ear emergence and flowering, leaf samples were collected at four time points and checked for Rcc by species-specific quantitative PCR to refit visual assessment. Results from the first season show a rapid increase in fungal DNA content within a few days in bacteria inoculated and control plants.

In addition to field experiments, laboratory and greenhouse-based work allows infection processes of RLS to be studied under controlled conditions. A common obstacle, however is the consistent production of conidia in culture. We developed a protocol for reliable conidiation to facilitate experimental reproducibility and further optimized conidial germination. Conidiation was observed in all tested strains, however, sporulation intensity varied considerably among isolates. Harvested conidia were viable and successfully infect detached barely leaves.

Partners

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Impact of Bacterial Seed Coating on Disease Development and Field Performance of Barley

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Depending on the severity of the infection and environmental conditions fungal diseases can reduce barley yield by up to 50 %. Priming for improved fungal resistance enables barley to respond stronger and faster to pathogen attack (Wehner et al., 2019).

We have previously identified primable and non-primable genotypes in a set of 200 spring barley accessions (IPK-SB224) under greenhouse conditions (Matros et al., 2023). To validate priming effects, 10 selected barley accessions were analysed in two-year field experiments. As a priming approach seed coating was applied with 108 CFU/g seed of two *Bacillus* species: FZB42-Rif *(B. velezensis)* and ABi11-Rif *(B. pumilus)*, or with H_2O in the control treatment. Primed seeds were sown in three replications in a randomised complete block design. Several agronomical important traits, such as plant developmental stage, plant height, grain yield and thousand grain weight (TGW) were scored. The estimation of soil coverage and disease symptoms of leaf rust and powdery mildew was carried out both, visually and with multispectral measurements of unmanned aerial vehicles (UAV). ANOVA revealed significant effects ($p<0.001$) of the priming treatment for TGW. Additionally, the priming with ABi11-Rif *(B. pumilus)* significantly ($p<0.001$) decreased the susceptibility of barley to leaf rust and powdery mildew. The effects of treatment with FZB42-Rif *(B. velezensis)* on symptoms of both diseases depended on the year. Fourteen weeks after sowing, Box PCR fingerprinting confirmed the presence of the applied bacteria in the rhizosphere of the primed plants, but not in the control plots. This demonstrated that the bacteria were capable of colonising the roots and surviving in the rhizosphere until the grain developmental stage. Our final aim is to develop molecular markers to select for primable genotypes in barley breeding programs.

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Bacterial quorum sensing molecule functions as postbiotics but the effects are dependent on substrate

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The use of postbiotics, defined as metabolites produced by microorganisms, represents a promising approach in sustainable agriculture. Their application is often more reliable than the use of living microorganisms.

In this study, we investigated the impact of the N-acyl homoserine lactone (AHL), *N*-hexanoyl-L-homoserine lactone (C6-HSL) on two spring barley genotypes, Golden Promise and BCC436. Both genotypes were already shown to respond to AHL treatment. We assessed plant performance, susceptibility to *Blumeria graminis f. sp. hordei (Bgh)*, and treatment-induced changes in the bacterial community of the rhizosphere.

Our results indicated that barley responses to C6-HSL are genotype-dependent and further modulated by the microbiome. In addition, the root-specific transcription factor MYB72 seems to be involved in the response, triggering induced systemic resistance (ISR) to *Bgh*. Although AHL treatment induced shift in the bacterial community, no individual taxa were identified as specifically responsive to C6-HSL treatment. This suggests a general but subtle restructuring of the microbial community.

Overall, C6-HSL application induced physiological changes in barley and triggered a top-down effect on the rhizosphere microbial community

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ROS1, DML3 and DME in Human Cells – A Side-by-Side Evaluation of Expression and Glycosylase Activity

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DNA glycosylases initiate base excision repair (BER) and, in plants, specialized glycosylases such as ROS1, DML3 and DME are central to active DNA demethylation. Although their enzymatic properties are well characterized in plant systems, it remains unclear to what extent these enzymes retain catalytic activity in a human cellular context. Here, we transiently expressed ROS1, DML3 and DME in human cells and assessed their glycosylase activity using a biochemical assay with defined oligonucleotide substrates. We demonstrate that plant glycosylases retain their catalytic function under heterologous expression conditions when transiently transfected in human cell lines. Our comparative analysis suggests that ROS1 displays the most robust activity in this assay. Ongoing work aims to determine how domain architecture and other structural determinants contribute to enzyme performance in human cells, providing a basis for future optimization and potential integration into targeted epigenome-editing approaches.

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Using Cas9-fused exonucleases to engineer disease-resistant and biofortified cis-genic tomato varieties

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The fusion of 5'-exonucleases to the Cas9 endonuclease (Cas9-Exo) significantly enhances homology-directed repair in plants, enabling the scar-free integration of several kilobases in both transient and stable assays (Schreiber *et al.*, 2024). This technology extends the scope of gene editing well beyond short DNA changes. Within the *INNO-TOM* project, we aim to exploit this innovative tool to develop tomato varieties with beneficial traits for both growers and consumers. The first approach focuses on engineering resistance to two tobamoviruses. We aim to replace the susceptible tm-2 allele with the dominant *Tm-2*^{2} allele, which encodes an NLR receptor that confers resistance to the Tomato Mosaic Virus (ToMV). We conducted transient assays showing that exchanging only the LRR region from *Tm-2*^{2} is sufficient to enable interaction with the viral movement protein (MP), thereby triggering a hypersensitive response (HR). A second virus, the Tomato Brown Rugose Fruit Virus (ToBRFV) has recently overcome Tm-2^2-mediated resistance, threatening tomato production in many countries. Screening of *Tm-22* mutant variants reported in the literature demonstrates that additional amino acid changes in the LRR domain promote recognition of the ToBRFV-MP by the receptor, resulting in HR. These mutations are being incorporated into our Cas9-Exo construct design for targeting the *tm-2* gene. Employing precise gene editing may accelerate the breeding of tomato varieties and avoid the linkage drag associated with the *Tm-2*^{2} locus obtained through classical breeding. The second trait focuses on increasing the anthocyanin content in tomato fruits. These purple-colored compounds provide antioxidant benefits to consumers and enhance post-harvest fruit longevity. We will employ Cas9-Exo to insert fruit-specific promoter sequences to drive the expression of R2R3-MYB transcription factors, which are key regulators of the anthocyanin biosynthesis pathway. By using DNA sequences already present within the tomato genome, cis-genic purple tomatoes can be generated, bypassing the stringent regulations applied to the *trans*-genic counterparts.

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Phenotyping methylation-compromised beets in the framework of the DPPN

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Epigenetic modifications, such as DNA methylation, play a crucial role in plant development and stress response, but remain underexplored in crop breeding. In this study, we will investigate how DNA methylation alterations may influence plant phenotypes of sugar beet *(Beta vulgaris ssp. vulgaris)* under abiotic stress and control conditions.

In the EpicBeet project, we have generated a range of DNA methylation-compromised beets and generated sequencing data for many of those. So far, among other tasks, we have optimized DNA sequencing and bioinformatics analysis to detect DNA methylation from long reads generated with Oxford Nanopore Technologies [1] and curated transposable elements of the sugar beet genome [2]. Based on these preliminary analyses, we have identified alterations in DNA methylation and numerous TE polymorphisms across the DNA methylation-compromised mutation lines.

However, to fully understand the functional implications of these DNA methylation alterations, it is essential to correspond them with actual plant morphology and phenotypes, such as shoot structure and leaf morphology. Within the framework of the German Plant Phenotyping Network (DPPN), we are currently phenotyping 150 EpicBeet plants, including a msh1 stop mutant and three different ddm1 point mutants under control, drought and salt conditions. This is done in the phenotyping platform Screen-House at Forschungszentrum Jülich. Here, we outline the setup and progress of our currently running experiment.

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Schmidt N, Maiwald S, Mann L, Heitkam T, BeetRepeats: reference sequences for genome and polymorphism annotation in sugar beet and wild relatives. BMC Res Notes 2024, doi: 10.1186/s13104-024-06993-4

Stability and heritability of DNA methylation patterns in sugar beet – impact on phenotypic plasticity, influence on TE activity and potential for beet breeding

N. Schmidt[1], J. Qian[1], L. Mann[1], T. Heitkam[1], M. Wulfhorst[2], P. Viehöver[2], B. Weisshaar[2], D. Holtgräwe[2], V. Vilperte[3], B. Schulz[3]

Although epigenetic modifications are causal to some plant phenotypes, epigenetically informed selection is not yet integrated in modern breeding. Potentially, DNA methylation accounts for the lack of heritability observed after genomic selection. However, only regions with stably inherited DNA methylation are useful for epigenomic selection. As the DNA methylation's stability and heritability have been investigated only scarcely, and solely for model plants, the fundamental basis for epigenetic crop breeding is still lacking. On top, as perturbance of DNA methylation is often followed by transposable element (TE) mobilization, it will also yield genetic variation.

In the EpicBeet project, we focus on the biannual crop sugar beet *(Beta vulgaris ssp. vulgaris)*. Its genome is diploid and therefore comparably small, has an annotated reference genome sequence [1], and has highly methylated [2], well-characterized repeats [3, 4]. To study epigenetic heritability, we focus on sugar beet mutants with perturbed DNA methylation. Overall, we aim to provide clear recommendations for the setup of epigenetic breeding lines as well as to generate fundamental, new information to better understand the heritability and stability of DNA methylation and its impact on the TE landscape in the sugar beet crop.

In the first project phase, we have screened more than 12,000 EMS-mutagenized plants to identify mutants in the hypomethylation-associated candidate genes *Decrease in DNA methylation (DDM1), Methyltransferase 1 (MET1)* as well as in the hypermethylation-associated gene *MutS Homolog 1 (MSH1)*. This screening resulted in a pool of seven different STOP and 61 different point mutations. For some of them (one STOP and fifteen point mutations) it was possible to generate homozygous mutant as well as epihybrid states.

Apart from generating material, we now have characterized the many mutants as well as the corresponding parental plants on a phenotypic, molecular and (epi-)sequence level using Oxford Nanopore Technologies (ONT) and enzymatic methyl-sequencing. To date, Illumina short read and methyl-sequencing data have been generated for 34 (epi)genotypes, whereas 26 were ONT-sequenced. To streamline the computational analyses, we have advanced the technology involved in generating ONT-based assemblies of beets as well as using them to infer DNA methylation and presenting the sugar beet reference methylome [2]. We have also curated our beet repetitive DNA reference libraries [4] and screened the sequenced sugar beet lines for TE polymorphisms. To better evaluate associated phenotypes, we are currently phenotyping 150 EpicBeet plants in the DPPN phenotyping facilities.

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A catalogue of recently active transposable elements in DNA methylation-compromised sugar beets

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Transposable elements (TEs) play a pivotal role in shaping eukaryotic genomes by moving within the genome or creating new copies that integrate into different loci. This process, known as (retro)transposition, can drive genetic and phenotypic diversity by modulating gene expression. Such genomic plasticity offers opportunities for advancing crop breeding programs. However, TEs can also pose a threat to genomic stability, prompting hosts to suppress TE activity by e.g. DNA methylation. Exploring the balance between TE activity and host control provides insights into leveraging their potential for agricultural innovation.

In the EpicBeet project, we are focussing on sugar beet *(Beta vulgaris subsp. vulgaris)*. Its genome consists of approx. 16% TEs [1], of which we have created a curated database [2]. To understand which TE families are predominantly mobilized in sugar beet, we screened ethyl methanesulfonate (EMS)-mutagenized sugar beets (Beta vulgaris subsp. vulgaris) for methylation-deficient mutants. These mutants were examined and are intended to be followed across generations to study both, the impact of EMS treatment and the impact of altered DNA methylation on TE mobility in sugar beet. Here, we present the TE polymorphisms in five of our sugar beet mutants. For instance, in our msh1-STOP mutant, we detected 1843 TE polymorphisms. Most of these (76%) correspond to miniature inverted-repeat transposable elements (MITEs), highlighting an unexpectedly large role of these small, non-autonomous TEs. In the reference genome, MITEs are highly methylated (in particular in the CG context; [3]), indicating that they are usually silenced. We are now exploring DNA methylation shifts in the sequenced lines. Furthermore, we will trace the proximity of the new TE insertions to genes and obtain an overview of mobile TEs in the sugar beet genome, including their origin/donor and integration/acceptor sites.

We conclude that MITEs are highly mobilized in sugar beet genomes and screening their activation may provide a shortcut in assessing genomic divergence. Finally, our findings highlight the crucial role of DNA methylation in TE regulation and demonstrate how its perturbation can drive genetic variability, offering new avenues for epigenetic breeding and the improvement of sugar beet cultivars.

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Comparative 5mC methylome analysis of DDM1-deficient mutants of Beta vulgaris ssp. vulgaris

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Epigenetic modifications, such as DNA methylation, contribute to phenotypic plasticity and shape diverse plant traits. Thus, epigenetic factors are fundamental drivers in adaptation to environmental stresses. Nonetheless, DNA methylation and its maintenance mechanisms may differ between species and are not well understood in non-model plants. Therefore, the potential of epigenetic breeding approaches in agriculturally relevant crop plants remains to be deciphered.

In *Arabidopsis thaliana (Ath), Decrease in DNA Methylation 1 (DDM1)* encodes a chromatin remodeller that mediates histone variant exchange and thus controls access to the DNA for enzymes that are involved in DNA methylation maintenance processes. It is associated with the inducible activation of defence-related genes [1] and the stimulation of transposable element (TE) silencing [2]. In *Athddm1* knockout mutants, the DNA methylation maintenance does not function properly and the mutant phenotype is characterised by genome-wide hypomethylation [2]. Similar to Ath, *DDM1* is a single copy gene in sugar beet *(Beta vulgaris ssp. vulgaris, Bvu)* and has two predicted helicase domains. In the TILLING-derived sugar beet *ddm1*-stop mutant (W312*), the stop-mutation is located within the first helicase domain, resulting in its truncation and the loss of the second helicase domain. While the heterozygous mutants are phenotypically indistinguishable from the wild type, homozygous mutants exhibit a restricted growth vigour. The homozygous mutants grow slower and remain tinier compared to the wild type. A few weeks after germination and the formation of a leaf rosette, the plants display a lethal phenotype. Besides phenotypic evaluation, we analysed the cytosine methylomes of homozygous mutants and the segregated wild types.

To detect 5-methyl cytosine (5mC) within the CG, CHG, and CHH context, high molecular weight genomic DNA was isolated from leaf *tissue of ddm1*-mutant and wild type plants and subjected to Oxford Nanopore Technology (ONT) sequencing. The ONT long-reads were mapped to a genotype-matched genome assembly, followed by the cytosine methylation detection by DeepSignal3/Unimeth. The genome-wide 5mC methylation patterns and gene/repeat-specific methylation profiles were analysed with 5mCartograph.

The *Bvuddm1* mutant displays genome-wide hypomethylation within symmetric sequence contexts (especially in CHG) compared to the segregated wild type plants. Centromeric regions depict the strongest hypomethylation. In contrast, cytosines within the CHH context are locally hypermethylated in satellite repeat-rich regions, while being hypomethylated around the centromeres. Gene-specific cytosine methylation profiles remain relatively stable, whereas repeat-specific profiles are characterised by hypomethylation within the CG and CHG context.

The results indicate that *DDM1* in sugar beet contributes to DNA methylation pattern maintenance, especially in symmetric sequence contexts in repeat-rich regions. This is congruent with results from studies on *A. thaliana* and represents the basis for future research.

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Mapping the Path to Embryogenesis: Standardization and Chemical Enhancement in Canola MS Cultures

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Background and Objectives
The reprogramming of canola microspores (MS) into embryo-like structures (ELS) is a highly sensitive developmental switch. In EpiHap, we aimed to characterize the morphological transitions during this process using high-throughput automated microscopy. By establishing a robust analytical framework, we sought to define a developmental timeline and evaluate the capacity of chemical reprogrammers to modulate and enhance ELS formation.

Methodological Optimization
Initial experiments identified mechanical stress and minor temperature fluctuations as critical disruptors of MS reprogramming, often resulting in impaired responses or the formation of atypical multicellular structures. Consequently, a standardized workflow was developed to stabilize the culture system. This included high-frequency time-lapse imaging in multiwell formats, allowing for the precise monitoring of viability and morphological progression under strictly controlled conditions.

Morphometric Results and Timeline
High-to-medium time resolved imaging revealed that while morphological changes remain minimal during the first six days post-isolation, ELS development accelerates significantly thereafter. A key finding was the “expansion-collapse” dynamic: during heat shock, the MS population undergoes a rapid sequence of expansion and subsequent collapse, which directly correlates with a decline in viability. Based on these data, a developmental progression timeline was established:

- 8 Hours: Peak of heat-induced expansion and onset of rapid collapse.
- Day 8: Final collapse of the population committed to the pollen pathway.
- Day 9: Appearance of the first distinct morphological markers of successful ELS reprogramming.

Chemical Modulation
The optimized protocol was applied to screen a selected set of chemical reprogrammers. Several compounds were identified that significantly increased ELS frequency compared to the standard heat-shock baseline. Experiments using MS fractions obtained by isopycnic centrifugation further revealed type-specific response levels, suggesting that these chemicals act on specific microspore subpopulations.

Conclusion: This study provides a quantitative basis for understanding early ELS development. The established timeline and the identification of chemical enhancers offer significant potential for advancing molecular understanding of underlying signaling cascades, optimizing doubled haploid production and improving the efficiency of canola breeding programs.

Keywords: Canola *(Brassica napus)*, Microspore Embryogenesis, High-Throughput Automated Microscopy, Time-Lapse Imaging; Chemical Reprogramming; Doubled Haploid Technology

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Progress on the induction of apomictic processes in barley

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Apomixis represents a transformative shift in agricultural biotechnology, offering a mechanism to produce seeds containing clonal embryos. By bypassing the genetic recombination inherent in sexual reproduction, apomixis allows for the transgenerational fixation of elite genotypes, maintaining hybrid vigor without the logistical complexities of traditional breeding schemes. To engineer an apomictic system in a cereal model like barley *(Hordeum vulgare L.)*, three distinct developmental transitions must be synchronized: apomeiosis (the bypass of meiosis), parthenogenesis (fertilization-independent embryo development), and autonomous endosperm formation.

While apomeiosis and parthenogenesis have been successfully induced in several species -often through site-directed mutagenesis of meiotic genes and the ectopic expression of BABY BOOM (BBM)-LIKE factors in the egg cell (Reed et al. 2025)- autonomous endosperm development remains the primary bottleneck. In *Arabidopsis*, this process is strongly regulated by epigenetic mechanisms (Pakaj et al. 2024, Figueiredo and Sharma 2025): H3K27me3 (trimethylation of lysine 27 on histone H3) governs initiation and expansion, while DNA methylation/demethylation pathways (mCG) controls cellularization.

The SEEDMAKER project addressed this challenge by integrating transcriptomic data with targeted genetic modifications and epigenetic remodeling. Utilizing RNA-seq on ovule and pericarp tissues dissected at five stages of development (before and after fertilization) and four tissue categories (including the female gametophyte/endosperm, integuments/nucellus, chlorenchyma and pericarp), we have identified over 18,000 differentially expressed genes (DEGs). These DEGs encompass pathways involving auxin signaling, carbohydrate and sucrose metabolism, and lipid degradation. We identified candidate genes for autonomous endosperm development in Arabidopsis and their putative barley orthologs, focusing on PRC2 genes and barley-specific histone 3 modifiers for tissue-specific manipulation of epigenetic marks. By leveraging promoters from these highly expressed, tissue-specific genes, and targeting genes having complementary roles in chromatin-based gene regulation, the project aims to modulate the depletion of repressive epigenetic marks and trigger autonomous endosperm formation.

In order to create a barley plant featuring apomeiosis and autonomous embryo formation, we used Cas9-mediated site-directed mutagenesis of the *HvREC8, HvPAIR1* and *HvOSD1A* as well as *HvOSD1B* genes that are combined with a parthenogenetic barley line capable of fertilization-independent embryo formation by egg cell-specific expression of *BBM*.

The final biotechnological milestone will involve the strategic hybridization of these lines capable of endosperm formation with apomeiotic and parthenogenetic barley mutants, culminating in an engineered, fully functional apomictic cereal.

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Integrative Analysis of Late Blight Resistance in Diverse Potato Germplasm

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Background
Cultivated potato *(Solanum tuberosum L.)* faces pathogenic force from rapidly adapting late blight strains *(Phytophthora infestans* (Mont.) de Bary). This has revealed the limitations of single dominant resistance genes, making the traditional *Solanum demissum* R1–R11 genes insufficient for sustainable breeding. However, various plant genetic resources offer promising opportunities to uncover novel resistance determinants.

Objectives
This project aims to achieve durable resistance by integrating functional genomics and applied breeding.

In detail, we will identify loci associated with resistance against late blight in the POMORROW Core Collection (PCC) and develop KASP markers for the most interesting loci. This will be complemented by the construction of a high-resolution genetic map and the identification of resistance alleles in segregating populations. Furthermore, functional genomic approaches will contribute to unraveling the underlying resistance mechanisms.

Material
Genetic mapping started on four segregating populations from the JKI resistance pre-breeding program. Tetraploid potato breeding clones carrying late blight resistance in the absence of known R genes, indicating the presence of alternative mechanisms or novel loci, have been established through introgressions from *Petota* accessions. The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) is currently assembling the PCC from 6,358 ex situ gene bank accessions. During 2027 and 2028, association genetic analyses of leaf and tuber blight resistance will be conducted on 150 clonal PCC accessions per year. Infection assays will use a standardized field isolate mix. Pathotypes will be characterized using an R gene differential set as well as sequencing techniques.

Methods
Phenotyping for late blight resistance will be conducted in two years in an alpha lattice design in five biological replicates, combining visual disease severity scoring on mini-tubers and leaves (Blossei et al. 2021) with field-based estimates of disease progression. Resistance-associated loci will be identified through association mapping and linkage mapping, depending on the considered genetic material. Parental clones of fine mapping populations will be long-read sequenced to provide reference haplotypes for the interpretation of short-read data from progenies. Candidate alleles will be prioritized through in silico expression analyses (Bonthala & Stich 2024), with the most promising genes subjected to time-course expression analysis and functional validation.

Current status
The phenotypic and molecular-genetic characterization of two segregating populations from the JKI resistance pre-breeding program has commenced. A preliminary experiment with one biological replicate per progeny indicates pronounced transgressive segregation for mycelial growth as the disease symptom under investigation. In the two populations examined so far, around one-third of the progeny showed estimated genetic values that exceeded those of the parental clone exhibiting favorable trait expression. Clonal propagation is scheduled and linkage mapping analysis will be performed once the sequence data have been provided.

Partners
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Self-compatibility in early generations of a diploid potato Nested Association Mapping (NAM) population

V. Wani[1], B. Stich[1], R. Gäbelein[1]

Diploid F_1 hybrid true potato seed breeding is an emerging strategy with the potential to transform global potato breeding and cultivation. Major efforts currently focus on identifying elite diploids and dihaploids to develop highly homozygous inbred lines. However, self-incompatibility remains a major barrier in diploid germplasm, requiring introgression of the dominant S-locus inhibitor *(Sli)* gene to enable self-compatibility. Even after overcoming this constraint, severe inbreeding depression and the accompaning reduced fertility limit the development of inbred parents. While nuclear genetic causes of inbreeding depression are well studied, less attention has been given to the role of cytoplasmic background and nuclear–cytoplasmic interactions with respect to fertility and self-compatibility during inbred line development.

Two NAM populations were developed by crossing diverse diploid founders with two near-homozygous *Sli* donors, M6 and 9H27-1, as common parent. Cytoplasmic diversity and its relationship with fertility and self-compatibility in early inbreeding generations was analysed using diagnostic markers T, S, SAC, A, and D (Hosaka and Sanetomo 2014). Fifteen founders carried D-type cytoplasm, three W and P-type each. The *Sli* donor M6 (Leisner et al. 2018) possessed W-type cytoplasm, whereas the second used *Sli* donor 9H27-1 (Hosaka and Sanetomo 2020) carried P-type cytoplasm, resulting in breeding populations with D, W, and P-type cytoplasmic backgrounds.

Twenty-seven F_1 populations carrying a heterozygous *Sli* allele were developed. Although all F_1 plants were expected to be self-compatible, several populations failed to set berries after self-pollination. Pollen viability assessments confirmed viable pollen in all tested F_1 plants. Nevertheless, strong differences in self-compatibility were observed, with some crosses producing uniformly incompatible progenies despite viable pollen. Compatibility varied between *Sli* sources and crossing direction. Crosses involving M6 yielded more self-compatible F_1 populations than those with 9H27-1. Moreover, using *Sli* donors as female parents resulted in higher compatibility frequencies than when used as pollen donor. Subsequent selfing produced 1,532 F_2 and 695 F_3 individuals, advanced through single seed descent. Pedigree was a major factor influencing fertility and vigour in inbred line development.

These results suggest that self-compatibility in diploid potato is not only determined by the presence of *Sli*, but also cytoplasmic type combined with pollen fertility assessments alone does not fully explain observed compatibility differences. The observed directional patterns instead suggest that nuclear background and potential nuclear–cytoplasmic interactions may modulate self-compatibility during early inbreeding. Incorporating cytoplasmic information into diploid breeding pipelines could therefore improve parental selection and enhance hybrid potato development efficiency.

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Phenotypic assesment of potato drought tolerance and responsiveness to arbuscular mycorrhiza

H. Rohwedder[1], A. Fernie[1], C. Gutjahr[1], K. Köhl[1]

Potato is one of the most important staple crops, but is not drought-tolerant. Breeding cultivars with improved drought tolerance is constrained by the low genetic variability of modern potato cultivars and the laborious screen for yield under arid conditions. Inoculation with arbuscular mycorrhiza fungi (AMF) can improve the water uptake from the soil and thus enhance yield stability during drought events. However, there is limited knowledge about the interaction of different potato accessions and AMF and their effect on drought tolerance. In the context of the POMORROW consortium, we investigate the drought tolerance and the interaction with AMF in a potato core collection(PCC) that represents the genetic variability of potato accession stored in the German Genebank at the IPK. In a bigbag system with automatic drip-irrigation, we are phenotyping the accessions of the PCC to identify genotypes with high drought tolerance and AM responsiveness. Replicates of each genotype receive either one of the four treatments: optimal water supply without (C-) or with AMF (C+) or reduced irrigation without (S-) or with (S+) AMF. Shoot development is automatically phenotyped with two Phenospex Planteye F600 multispectral 3D scanners, that measured morphological and multispectral parameters throughout the experiment. Based on the phenotypic data of the first subpopulation screened in 2025, we identified genotypes with high drought tolerance and/or high responsiveness to AMF. To confirm these findings, the selected genotypes will be included in the next experiments. This procedure will be continued the next three years to gather a collection of drought tolerant and AMF responsive genotypes as potential breeding parents. Furthermore, the phenotypic data and genotypic date of the PCC will be used for GWAS to identify the genetic basis for drought tolerance and AM responsiveness.

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Developing strategies for transgene-free genome editing in potatoes

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Potato breeding is challenging due to the autotetraploid, highly heterozygous genome of cultivated varieties, which makes the introgression of valuable traits slow and unpredictable. This project aims to establish robust, transgene-free genome editing strategies that enable the rapid generation of elite potato lines carrying homozygous knock-out mutations or precisely integrated cis-genes without integration of foreign DNA. A central focus lies on the development of broadly applicable methods that function beyond model cultivars and can be transferred to commercially relevant and transformation-recalcitrant genotypes.

One strategy is based on the delivery of pre-assembled CRISPR/Cas9 ribonucleoprotein complexes into protoplasts of elite lines, followed by plant regeneration. Protospacers were designed to cover all alleles of selected target genes and validated in vitro. Editing efficiencies will be assessed using a GUS reporter system and endogenous genes involved in tuber flesh colour. Molecular analyses of regenerated shoots will include fragment length analysis and sequencing to distinguish homozygous, heterozygous and chimeric events. In the future, the system will be used to integrate cis-genes e.g., *rpi-blb2*, mediating late blight resistance.

As a second strategy, a grafting-based approach is being established to enable editing of otherwise non-transformable elite varieties. In this system, CRISPR/Cas9 components are expressed in a genetically accessible donor cultivar (i.e., Desirée) and delivered across a graft junction into the recipient plant via the phloem. Cas9 and guide RNAs are fused with different mobility-enhancing RNA tags (3'FT (Ellison et al., 2020), 3'SP6A, and 3'tRNA (Yang et al., 2023)) to promote long-distance transport and entry into meristematic tissues, including the shoot and stolon apical meristems, which give rise to tubers. First transgenic donor lines carrying tagged editing constructs were generated and characterized for expression prior to grafting onto elite rootstocks. Developing stolons and emerging tuber-derived shoots will be subsequently analysed for the presence of mobile CRISPR components and for targeted mutations in the genes of interest. Initial experiments confirmed stable expression of tagged constructs in donor plants and successful graft union formation, providing the basis for systematic evaluation of phloem-mediated genome editing in recipient genotypes. Now grafting onto elite lines and subsequent analysis will follow.

Together, these approaches aim to provide innovative and broadly applicable, transgene-free genome editing workflows, enabling precise trait modification and facilitating the improvement of genetically complex and previously inaccessible elite breeding lines.

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Building a Genetically Representative Potato Core Collection for European Breeding Using Multi-Objective Optimisation

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Background
German and European potato breeding faces intensifying pressure to deliver cultivars resilient to abiotic stress, biotic pathogens, and reduced-input production while sustaining yield and quality (Kikuchi et al., 2015; Obidiegwu et al., 2015). Modern cultivars often display limited tolerance to these stressors due historical breeding prioritisation of productivity over broader stress resilience (Kikuchi et al., 2015). The genetic basis for stress resilience is frequently concentrated in underutilised genebank germplasm, yet large collections remain difficult to phenotype comprehensively and harbour substantial redundancy (Odong et al. 2013). The POMORROW project addresses this bottleneck by developing an experimentally tractable, genetically representative potato core collection that captures the major diversity strata of the Groß Lüsewitzer Potato Collections (GLKS; 6,300+ accessions) for efficient phenotyping, pre-breeding, and resource sharing among European breeding partners.

Methods
For a basic core collection, approximately 2,500 clonal accessions from IPK's cultivated and Andean potato collections were genotyped using genotyping-by-sequencing (GBS). Population genetic structure was characterised via PCA and identity-by-state (IBS) relationships. Core collection optimisation was conducted with CoreHunter3 using dual objectives: maximising mean pairwise genetic distance (MD) and entry-to-nearest-neighbor distance (EN) to broaden diversity capture and minimise redundancy (De Beukelaer et al. 2018). We performed repeated independent optimisation runs to evaluate stability and derive a robust candidate list.

Results
Quality filtering retained ~45,000 high-quality SNP markers. Multi-objective optimi-sation converged stably, consistently recovering a consensus subset spanning the major IBS structure across collections. In a cohort-1 proof-of-concept (n=200), the core exhibited higher MD and EN relative to the full germplasm set. SNP presence strongly overlapped between core and non-core groups: 34,964 SNPs (75.7%) were shared across all groups, while only 474 SNPs (1.1%) were non-core-specific, indicating near-complete capture of common variation with minor loss of rare alleles only.

Conclusions
SNP markers combined with multi-objective optimisation provide a transparent, auditable pathway to constructing a potato core collection that balances diversity richness with operational tractability for breeding-relevant phenotyping. Building on the proof-of-concept, next steps are to (i) scale selection to the full clonal collection and extend it to seed accessions, (ii) quantify diversity retention with allele-capture curves, and (iii) integrating presence/absence variation (PAV) from the *Petota* super pangenome to capture structural variation and accessory genome content beyond SNP diversity. This core collection will serve as a foundational resource for accelerating German and European potato breeding while maintaining access to underexploited genetic variation for future resilience breeding objectives.

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Phenotypic assesment of potato drought tolerance and responsiveness to arbuscular mycorrhiza

H. Rohwedder[1], A. Fernie[1], C. Gutjahr[1], K. Köhl[1]

Potato is one of the most important staple crops, but is not drought-tolerant. Breeding cultivars with improved drought tolerance is constrained by the low genetic variability of modern potato cultivars and the laborious screen for yield under arid conditions. Inoculation with arbuscular mycorrhiza fungi (AMF) can improve the water uptake from the soil and thus enhance yield stability during drought events. However, there is limited knowledge about the interaction of different potato accessions and AMF and their effect on drought tolerance. In the context of the POMORROW consortium, we investigate the drought tolerance and the interaction with AMF in a potato core collection (PCC) that represents the genetic variability of potato accession stored in the German Genebank at the IPK. In a bigbag system with automatic drip-irrigation, we are phenotyping the accessions of the PCC to identify genotypes with high drought tolerance and AM responsiveness. Replicates of each genotype receive either one of the four treatments: optimal water supply without (C-) or with AMF (C+) or reduced irrigation without (S-) or with (S+) AMF. Shoot development is automatically phenotyped with two Phenospex Planteye F600 multispectral 3D scanners, that measured morphological and multispectral parameters throughout the experiment. Based on the phenotypic data of the first subpopulation screened in 2025, we identified genotypes with high drought tolerance and/or high responsiveness to AMF. To confirm these findings, the selected genotypes will be included in the next experiments. This procedure will be continued the next three years to gather a collection of drought-tolerant and AMF responsive genotypes as potential breeding parents. Furthermore, the phenotypic data and genotypic date of the PCC will be used for GWAS to identify the genetic basis for drought tolerance and AM responsiveness.

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Providing access to state-of-the art phenotyping infrastructures

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The German Plant Phenoytyping Network (DPPN) was established in 2012 in the context of the BMBF-funded DPPN-project by Forschungszentrum Jülich (FZJ), Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben and the Helmholtz Munich (HMGU) to develop, establish and extend state-of-the-art infrastructures for plant and breeding research. DPPN has implemented a world-leading research infrastructure that addresses the “phenotyping gap”, which has emerged in the last decades due to rapid developments in genotyping technologies while phenotypic analysis has been lagging behind. To address the “phenotyping gap”, DPPN established a portfolio of state-of-the-art infrastructures for quantitative plant phenotyping of different crops under various environmental scenarios **https://dppn.plant-phenotyping-network.de/**.

After the end of the DPPN-project, the DPPN partners formed in 2018 the association DPPN e.V. to continue the partnership and enable user access to the plant phenotyping installations, techniques and methods build by the DPPN partners. The infrastructures are accessible to all relevant German users (mostly researchers from university) in a cost-effectively manner. Within the pilot project “DPPN-ACCESS” (01.01.2022-31.12.2023) the process for the access was drawn up and access was granted on basis of a simple application procedure. Within these two years 14 applications have been approved.

In order to ensure the implementation of further external user projects in the longer term, the follow-up project “DPPN-ACCESS 2.0” (01.01.2024-31.12.2028) was acquired. In the meantime, three calls have been launched, and 36 applications have been approved. A new call for applications is currently open, with a deadline for submission until April 14 **https://dppn.plant-phenotyping-network.de/Access_Calls**. Further calls will be launched approximately every six months. The poster presentation will summarize the current status of “DPPN-ACCESS”, describe the available facilities, and highlight selected access experiments conducted at FZJ, IPK and HMGU so far.

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Promoting the use of plant phenotyping facilities and technologies @ IPK

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Project aims and goals

The German Plant Phenotyping Network (DPPN) e.V. is a joint initiative of Forschungszentrum Jülich (FZJ), the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, and Helmholtz Munich (HMGU), established to address the persistent “phenotyping gap” in plant and breeding research. By developing and operating complementary, state-of-the-art phenotyping infrastructures, DPPN e.V. enables quantitative analysis of plant performance across crops and environmental conditions. Through the BMFTR-funded access programs DPPN-ACCESS and DPPN-ACCESS 2.0, these facilities are made available to external academic users via a transparent and low-threshold application process, fostering collaboration and maximizing the scientific impact of national phenotyping resources.

Access projects carried out at IPK plant phenotyping platforms

At the IPK, a total of 14 external user projects have been accepted since the pilot phase of DPPN-ACCESS, nine of which have already been conducted and successfully completed. In the most recent call (Call 2, 2025), four proposals targeting IPK installations were submitted and approved and the access projects will be performed in 2026.

Access to IPK infrastructures has been requested by seven different universities and research institutions, involving 27 team members (10 female, 17 male). Of the seven phenotyping installations offered by IPK, five have been utilized by users, with strongest demand for the whole-plant phenotyping platforms APPP-A and APPP-B as well as the rhizotron system in the IPK PhenoSphere.

Several IPK access projects have already resulted in substantial scientific output. The pilot-phase project Re-tiller (Martin Luther University Halle-Wittenberg) led to a peer-reviewed publication (Soleimani et al. 2025, Stress, doi:10.1016/j.stress.2025.100954), and a follow-up study (90-20-90-20) is currently in preparation. Also for the wheat PPPI project, several publications and master thesis are being prepared. Further examples of successful collaboration include the projects MenthaDro and MenthaOmics of the Julius Kühn-Institut (JKI), conducted on the APPP-A platform. In MenthaDro, 70 accessions representing more than ten Mentha species were phenotyped under controlled well-watered and drought stress conditions to assess shoot and root responses. In MenthaOmics, selected contrasting genotypes were analyzed using the same experimental setup, complemented by targeted metabolomics sampling. Data analysis for this second project is ongoing. The results from both studies will complement datasets from the JKI MenthaSens breeding project and form the basis of a joint publication.

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CRISPR/Cas9-Based Targeting of Tannin Biosynthesis Genes to Develop Zero-Tannin, Chilling-Tolerant Sorghum

XB. Knoblauch[1], S.M. Augustine[1], S. Windpassinger[1], R. Snowdon[1]

Sorghum bicolor (L.) Moench is a globally important cereal crop used for food, feed, fodder, and bioenergy production. Although sorghum exhibits superior drought tolerance compared with maize, its cultivation remains largely confined to tropical and subtropical regions due to its high sensitivity to chilling and frost. Expanding sorghum production into temperate environments is therefore a key breeding objective under climate change scenarios. A major limitation in breeding chilling-tolerant sorghum is the frequent genetic linkage between chilling tolerance loci and genes involved in grain tannin biosynthesis. Grain tannins are polyphenolic compounds that reduce palatability and nutrient bioavailability, rendering tannin-containing sorghum varieties unsuitable for most food and feed applications. Consequently, breeding programs often face a trade-off between chilling tolerance and grain quality.

In this study, we aim to generate chilling-tolerant, tannin-free sorghum lines by targeted knockout of the tannin biosynthesis genes *TAN1* and *TAN2* using CRISPR/Cas9 genome editing. Efficient genome editing in sorghum is challenged by its strong genotype dependency and recalcitrance to tissue culture–based transformation. To overcome these constraints, the first objective of this work is to establish an efficient and reproducible tissue culture and regeneration system for selected tannin-containing sorghum genotypes from an ongoing breeding program. Multiple explant sources and culture media compositions are being evaluated to improve regeneration efficiency while reducing labor intensity.

In parallel, advanced genome editing delivery strategies are being optimized, including transgene-free CRISPR/Cas9 ribonucleoprotein (RNP) delivery and viral vector-mediated approaches. These strategies aim to enable precise editing of tannin biosynthesis genes without stable transgene integration, thereby facilitating regulatory acceptance and accelerating downstream breeding applications. The successful development of chilling-tolerant, zero-tannin sorghum lines will provide valuable germplasm for temperate-adapted breeding programs and support the wider deployment of sorghum as a climate-resilient cereal crop. This work highlights the potential of genome editing and improved transformation platforms to overcome long-standing genetic bottlenecks in sorghum improvement.

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Lowering the carbon footprint of C4 systems: A multi-site life cycle assessment of sorghum and maize genotypes in Germany

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Introduction

Sorghum is a promising C4 crop gaining attention as a climate-resilient alternative under changing agro-climatic conditions in Central Europe. As a relatively low-input C4 crop, sorghum may be well suited to low-emission cropping systems, while its extensive root system may support soil organic carbon accumulation and carbon sequestration. This study presents a comparative life cycle assessment (LCA) of multiple grain sorghum genotypes cultivated in Germany, benchmarked against maize, the dominant C4 grain crop. We focus on greenhouse gas (GHG) emissions and climate change impacts associated with crop production.

Methods

Field trial data from eight locations across Germany (Berlin, Braunschweig, Quedlinburg, Groß-Gerau, Trebur, Schwarzenau, Moosburg, and Frankendorf) covering 18 environments across multiple growing seasons were analysed to capture spatial and temporal variability. A cradle-to-farm-gate LCA was conducted, including upstream and on-farm emissions, with emission factors derived from the ecoinvent database and N_2O emissions estimated using IPCC Tier 2 methodology. GHG emissions were calculated per unit area (kg CO_2-eq ha^{-1}) and per unit product (kg CO_2-eq kg^{-1} grain) for each genotype × location × year combination. The trials comprised commercial sorghum genotypes, sorghum test hybrids and parental lines, and commercial maize grown within or adjacent to the sorghum trials. Carbon footprints among genotype groups were compared using linear mixed-effects models.

Results and Discussion

Maize generally produced higher area-based emissions than sorghum (up to ~4 vs ~2.5 t CO_2-eq ha^{-1}), but higher yields resulted in similar average carbon footprint (CFP) of ~0.2 kg CO_2-eq kg^{-1} grain for both crops. Maize showed greater CFP variability, reflecting higher sensitivity to climatic conditions. Under favorable soils and sufficient water supply, maize outperformed sorghum due

to superior efficiency, as observed in Quedlinburg in 2023 and 2024. Whereas, under drought and poorer soil conditions, sorghum maintained more stable yield and lower CFP as observed in dry Bavarian sites between 2021 and 2023. In 2024, with sufficient rainfall, CFP values converged. In Brandenburg, sorghum consistently exhibited lower CFP on sandy soils, while under full irrigation, both crops performed similarly. Intermediate regions showed strong management effects, emphasizing the need for context-specific strategies. Sorghum test hybrids had significantly lower CFP than parental lines and commercial genotypes across environments, highlighting their potential for low-emission cropping systems.

Conclusion

Sorghum represents a viable alternative to maize in drought-prone regions and on suboptimal soils where maize yield stability is limited. Ongoing breeding progress further enhances sorghum's potential to reduce production-related emissions. The development of climate-resilient, site-adapted sorghum genotypes can support sustainable intensification and targeted breeding strategies under climate change.

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Transpiration dynamics of F1 hybrids and their parents provide insight into the response of sorghum to drought stress

M. Lulie[1], C. Seiler[1], A. Elkhouly[1], G. Wehner[1], A. Stahl[1], J. Klinger[2], S. Windpassinger[3]

Higher water use efficiency (WUE) is desirable for growing sorghum under increasingly dry growing conditions. As a C4 crop with high resource-use efficiency, Sorghum serves a model crop for studying transpiration efficiency and water use under drought. Although the performance superiority of hybrid varieties has been widely described, little is known about the magnitude of the heterosis effect for transpiration-associated traits.

Using a gravimetric deep phenotyping facility (Plantarray, Plant-Ditech), we monitored real-time water use, transpiration and biomass accumulation in 20 sorghum genotypes (ten hybrids, seven restorer lines, two mother lines and one commercial reference variety). Plants were grown in three replications, in a semi-controlled greenhouse under well-watered (soil water capacity of 70%) and drought stress conditions (WC of 25%), by watering based on pot weight. Continuous weight measurements every three minutes enabled tracking of transpiration throughout the whole experimental cycle and within individual days, allowing real-time dissection of genotypic responses to drought stress. As such, various transpiration traits including transpiration ratio, transpiration peak, daily and cumulative transpiration, and average transpiration per hour were monitored. In addition, dry weight and WUE were analyzed.

Results revealed significant ($p < 0.001$) genotypic variation in WUE and transpiration dynamics. In control treatment, genotypes showed higher daily total transpiration, cumulative transpiration, transpiration peak values, pre- and post-midday transpirations, and low WUE. Whereas, in the stress treatment genotypes showed high WUE combined with low daily and cumulative transpiration. Interestingly, in the stress treatment genotypes consistently showed low post-midday transpiration than pre-midday condition, which leads to those genotypes closing their stomata in order to conserve the available water.

Some genotypes maintained high WUE while sustaining biomass accumulation, whereas others adopted a water-conservation strategy by reducing stomatal conductance. Consequently, distinct genotype-specific patterns were observed allowing discrimination among genotypes based on their water consumption characteristics.

This study highlights the value of continuous, non-invasive phenotyping for deepening the understanding of water use patterns of diverse genotypes under drought stress. The observed variation in WUE and transpiration associated traits provides a foundation for breeding drought-tolerant sorghum varieties better adapted to water-limited conditions.

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Sorghum Pangenomics – Genome Assembly, Annotation, and Structural Variant Analysis

SorBOOM Agrobioinformatics

Kübra Arslan[1], An Nguyen-Hoang[1], Venkataramana Kopalli[1], Steffen Windpassinger[2], Andreas Stahl[3], Agnieszka A. Golicz[1]

Haplotype-resolved genome assemblies were generated from PacBio HiFi sequencing data using hifiasm, followed by error correction and scaffolding with the in-house developed noHiC pipeline. Genome annotation was conducted by benchmarking Tiberius against BRAKER3, trained with publicly available sorghum RNA-seq and protein datasets. Owing to higher annotation completeness, BRAKER3 was selected for downstream analyses. Repeat annotation has been completed for all assemblies, while genome annotation is currently ongoing and has been finalized for approximately half of the assemblies, with completed annotations showing an average completeness of around 95%.

Structural variant discovery was completed using an integrated framework combining assembly-based and read-based SV calling approaches. SV genotyping is currently ongoing, with multiple tools being benchmarked to establish a robust genotyping strategy across genotypes. Additionally, RNA samples were collected from all genotypes at the Gross-Gerau field trial during the booting stage, and RNA isolation and sequencing are scheduled for completion in the second quarter of 2026.

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A Digital Discovery Platform for Cereal Genetic Resources

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Background and Motivation
Crop genetic resources conserved in genebanks represent a unique repository of evolutionary and adaptive diversity, yet their systematic utilisation in research and breeding remains limited by fragmented genomic and phenotypic information. Recent advances in whole-genome sequencing, pangenomics, and data science now enable a transition from static collections to integrative, data-driven discovery systems.

Concept and Objectives
The Twin project establishes the conceptual and technical foundation for *digital twins* of cereal genetic resources. Focusing on barley *(Hordeum vulgare)* and wheat *(Triticum aestivum)*, Twin creates dynamic digital counterparts of genebank accessions that integrate genome sequences, pangenome context, phenotypes, and environmental metadata. These digital twins form a continuously evolving discovery platform that links conservation, functional genomics, and translational breeding.

Data Generation and Infrastructure
Twin will generate whole-genome sequence data for approximately 15,000 non-redundant barley accessions (~10× coverage) and 1,500 representative wheat accessions. The resulting variant, haplotype, and structural-variation datasets are integrated into existing barley and wheat pangenomes. FAIR-compliant data architectures, databases, browsers, and APIs provide sustainable access and interoperability with national and international infrastructures.

Data Science and AI-Based Analysis
Advanced bioinformatics and artificial-intelligence approaches form the analytical backbone of Twin. Deep-learning models and genomic language models are applied to genome annotation, regulatory-element inference, and variant-effect prediction, enabling improved functional interpretation at genebank scale. The resulting pan-regulome resources link sequence variation to gene regulation, expression, and phenotype.

Translational Case Studies
The utility of the digital-twin framework is demonstrated through targeted biological case studies. In barley, Twin investigates the genetic and metabolic basis of hordatine-mediated defence and natural variation in meiotic recombination landscapes. In wheat, integrative population-genomic and transcriptomic analyses identify adaptation, resistance, and reproductive-development genes relevant for breeding.

Impact and Outlook
Twin establishes a scalable, interoperable blueprint for digital genebank resources, transforming preserved diversity into an active, predictive research infrastructure. By coupling large-scale genomics with AI-driven analysis, the project advances Germany's leadership in plant genetic-resource digitalisation and provides a foundation for next-generation, data-informed breeding strategies.

Partners
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Cornwall

S. Wang[1], V. Ramirez[1], M. Pauly[1], S. Kusch[2], B. Usadel[2], K. Kafoutchoni[3], B. Stich[3], F. Röber[4], T. Bolduan[5], T. Prestrl[5]

Most agricultural residues are composed of lignocellulose, a complex, non-food plant biomass whose constituent sugars, once liberated, can be converted via microbial fermentation into renewable, carbon-neutral commodity chemicals. A major bottleneck in the valorization of lignocellulosic biomass is its inherent recalcitrance to degradation. Consequently, there is substantial potential to breed crops with improved lignocellulosic characteristics that reduce processing costs and energy inputs.

The Cornwall project exploits the extensive genetic diversity of maize to identify lines with favorable, altered lignocellulosic properties. In addition, causative single nucleotide polymorphisms (SNPs) are being identified to provide mechanistic insight into how specific gene variants confer these enhanced traits, enabling the transfer of this patentable knowledge to other grass species.

Genome-wide association studies (GWAS) of lignocellulosic traits conducted on a diverse European and American maize landrace panel (>300 accessions) revealed multiple quantitative trait loci (QTLs). Sequence analysis within these QTLs identified candidate SNPs which, based on haplotype analyses, contribute significantly to the observed variation in lignocellulosic composition. Functional validation of these SNPs is achieved through e.g. heterologous expression of the corresponding proteins and analysis of targeted plant knock-out lines.

To further expand maize genetic diversity, selected landraces were crossed with a Dent SC hybrid to generate four-way hybrids. These hybrids were subsequently characterized for their lignocellulosic chemotypic properties. The results demonstrate that the four-way hybrids substantially broaden the lignocellulosic trait space relative to the original landrace population.

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TEAM7 – Expanding the breeders' tool set for genome editing in barley and oilseed rape

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The enhancement of crop varieties is predominantly predicated on the utilization of genetic diversity that mainly derives from random mutagenesis. This process occurs spontaneously or is substantially intensified by the application of mutagenic chemicals or ionizing radiation. Notably, the novel aspect of genome editing is its ability to address specific genomic positions for genetic modification while the genetic background remains unaffected. In light of this paradigm shift, there are numerous opportunities to enhance the velocity of breeding progress and the precision of genetic modifications. In addition, the utilization of genome editing technology in the realm of plant breeding has also become more tangible in the European societal context. This development follows the presentation of draft amendments to the European legal directive on genetic engineering by the EU Commission and the agreement recently reached in the trilogue procedure. At present, RNA-guided Cas endonucleases derived from microbial CRISPR/Cas immune systems are considered to be the most effective tools for genome editing.

The objective of the TEAM7 project is to expand the spectrum of Cas endonucleases available for cultivated plants by methodically establishing alternative ones and to provide new possibilities for genetic modifications with increased precision. While the TEAM7 project is coordinated by the GFPi, the experimental work is being conducted at the KIT of the University of Karlsruhe (PI Holger Puchta), at the Leibniz University of Hannover (PI Jens Boch), and at the IPK Gatersleben (PI Jochen Kumlehn). Our task at the IPK is to enhance the capabilities of genome editing technology in barley and oilseed rape by employing enhanced variants of Cas12a as well as a novel Cas endonuclease generated by means of artificial intelligence (AI).

To this end, reliable protoplast systems for the pre-validation of genetic constructs and efficient methods of stable transgenesis for barley and oilseed rape have been established. In barley protoplasts, the employment of a temperature-tolerant LbCas12a variant and the AI-developed openCRISPR Cas9 has led to mutations within the target motifs. Further refinements of these endonucleases are underway with respect to promotors, coding sequences, nuclear localization signals and intron-mediated enhancement. The modularity and variability of the CasCADE vector system, developed in our laboratory, will prove instrumental in this endeavor. In addition to the experimental approaches currently being pursued, internships are offered by the academic partners to employees from the numerous breeding and biotech companies involved in the project, enabling them to familiarize themselves with the laboratory work associated with genome editing. In the second phase of the project, we envision the utilization of the developed Cas endonuclease variants to enhance the nitrogen use efficiency in both oilseed rape and barley.

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Wiki-how to – Benefit from the German ex-situ federal genebank

AE Backhaus[1]

Climate change and the degradation of natural habitats are forcing a fundamental re-evaluation of agricultural systems. As farming conditions shift, crops must deliver traits that were historically overlooked in breeding programs, such as new disease resistances, drought tolerance, and contributions to soil and ecosystem health. These traits have not been fostered in breeding thus far, and are largely absent from modern breeding pools. Genebanks are thus a reservoir of diversity for novel traits relevant to research and breeding. The genebank collection at IPK contains about 130,000 accessions of cereals, legumes, vegetables, and medicinal plants.

In an increasingly digitalized world, genebanks must rethink how they can leverage modern technologies to dismantle historical barriers and enable equitable seed access, beyond highly trained experts. Leveraging novel data sources, such as high thruput phenotyping and genotyping data, is only worthwhile if simple tools enable the use and benefit from this data for all genebank users. We here present our current advances and future visions for a fair and usable digital genebank.

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Activation of Exotic Wheat Genetic Resources for Disease Resistance

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The EXOGENE initiative aims to expand the genetic basis of wheat improvement by mobilizing underutilized and geographically distinct germplasm. The focus is on integrating wild relatives, alien introgression materials, and historically diverse wheat collections into a harmonized phenotyping framework for disease resistance.

A comprehensive *Triticum araraticum* collection comprising 285 genotypes was assembled by integrating a Turkish core collection with additional genebank accessions from worldwide origins. In parallel, a diverse panel of alien introgression, substitution, and addition lines derived from crosses with *Aegilops* spp. and other wild relatives was evaluated. Furthermore, a collection of historic and modern Australian wheat varieties was included to assess resistance alleles that evolved under geographically isolated breeding conditions.

All materials were screened for resistance to leaf rust and yellow rust under controlled conditions. Across panels, a wide and continuous phenotypic distribution was observed, ranging from high susceptibility to strong resistance. This confirms substantial exploitable diversity within and across genetic backgrounds. Detailed statistical analyses are ongoing.

To establish a genomic framework, the *T. araraticum* collection will be genotyped by sequencing to remove redundant or misclassified accessions and to define population structure. A genetically representative accession will be selected for de novo whole-genome assembly to enable high-resolution mapping and allele mining. Genotypic characterization of additional panels will support the identification and prioritization of resistance loci for downstream validation and pre-breeding.

The combined phenotypic and genomic efforts provide a structured basis for activating exotic genetic resources and translating novel resistance variation into wheat improvement pipelines.

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High-Resolution Genomics and Microphenomics of Nonhost Resistance to Rust and Mildew

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Wheat production is severely constrained by biotrophic fungal pathogens such as powdery mildew and yellow rust, which are highly adapted to the wheat immune system. In contrast, closely related cereal species often display effective resistance because pathogen virulence mechanisms fail to suppress their defense responses. Exploiting such nonhost and near-nonhost resistance remains a promising but methodologically challenging strategy for wheat improvement.

Building on our previous work, we expanded the genetic resolution of the barley diversity panel by integrating recently released whole-genome sequencing datasets (Jayakodi et al. 2024), resulting in a markedly higher SNP density than with earlier datasets. These data enabled genome-wide association analyses using both linear mixed models and machine-learning-based approaches. In parallel, we initiated a transition from single-reference analyses toward pan-genome-aware workflows and reference-free k-mer-based association mapping to capture better structural variation and presence–absence polymorphisms that are poorly represented in linear reference genomes.

In parallel, we further developed the microphenotyping approach for rust diseases to enable quantitative assessment of early infection stages under controlled conditions. Preliminary results indicate species-specific response patterns in wheat and barley that are not captured by conventional macroscopic disease scoring. This ongoing work aims to improve the resolution of early host-pathogen interactions in cereal nonhost resistance studies.

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BarleyCOPA – Computational inference of GxGxE interactions to identify climate-change resilient pathogen resistance in barley – Part F.

Sebastian Urzinger[1], Amira Boukherissa[2], Thomas Lux[1], BarleyCOPA Consortium, Klaus F.X. Mayer[1] 2, Aurelien Tellier[2], Manuel Spannagl[1]

The interaction of crop host genotypes with pathogen genotypes determines resistance of crops and the severity of epidemics by under a given climatic/environmental condition. These Genotype x Genotype x Environmental (GxGxE) interactions determine the resilience of future disease resistance in crops. To support the determination of Genome-to-Genome associations (GtoG or co-GWAS) between genetic diversity in barley (SNPs in the barley pan-genome, or Copy Number Variants; CNVs) and pathogen diversity (SNPs and CNVs) we constructed a genomic framework consisting of existing and emerging barley (pan-) genome data. This includes orthologous and synthenic relationships between all barley high-quality genome sequence assemblies, as well as a comprehensive analysis of gene copy number and gene presence/absence.

Partners

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Cereal (Pan-) Genomics

PREBreed

PROGRESS

p-epBAR

DRIVE

RYE HUB

INTEGRA

AIM4GEM

FABALOUS

BeetAdapt

BIMOTEC

Pest Protection

Res4StRes

BarleyCOPA

PrimedPlant-3

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501 Stability and heritability of DNA methylation patterns in sugar beet – impact on phenotypic plasticity, influence on TE activity and potential for beet breeding
N. Schmidt, J. Qian, L. Mann, T. Heitkam, M. Wulfhorst, P. Viehöver, B. Weisshaar, D. Holtgräwe, V. Vilperte, B. Schulz

502 A catalogue of recently active transposable elements in DNA methylation-compromised sugar beets
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503 Comparative 5mC methylome analysis of DDM1-deficient mutants of Beta vulgaris ssp. vulgaris
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510 Mapping the Path to Embryogenesis: Standardization and Chemical Enhancement in Canola MS Cultures
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POMORROW

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DPPN-ACCESS

SorBOOM

TWIN

Weitere Projekte