

Doi: 10.46793/MAK2026.067P

OVERVIEW OF PLANT GENETICS AND BREEDING IN THE ERA OF PANGENOMICS AND GENOME EDITING: CONTRIBUTIONS FROM OUR RESEARCH GROUP

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Abstract: Climate change intensifies abiotic stress and disease pressure on crops, while domestication has narrowed genetic diversity. Advances in high-throughput sequencing, pangenomics and genome editing enable discovery, validation and precise deployment of beneficial alleles from landraces and wild relatives. These approaches transform plant breeding into a predictive, sustainable discipline, illustrated here on barley and wheat resistance breeding.

Keywords: Gene isolation, Pangenome, CRISPR/Cas, Barley, Wheat, Resistance breeding

INTRODUCTION

Rising weather extremes, increased frequency of droughts, heat waves and floods, together with rapidly evolving pest and pathogen populations, represent some of the most serious challenges to global crop production in the 21st century. Climate change not only affects plant growth directly through abiotic stress, but also indirectly by altering the distribution, aggressiveness and life cycles of pathogens and pests. Yield instability and crop losses caused by diseases are therefore expected to increase, particularly in major cereal crops that form the backbone of global food security.

Plants are sessile organisms and cannot escape unfavorable environmental conditions. Instead, they rely on complex and tightly regulated genetic and epigenetic mechanisms that enable perception of stress signals, activation of defense responses and physiological adaptation. Over evolutionary timescales, natural selection has shaped extensive genetic diversity in wild plant populations. However, domestication and intensive modern breeding have narrowed the genetic base of many crops, creating genetic bottlenecks that limit adaptive potential.

For decades, plant breeding relied primarily on phenotypic selection and empirical crossing schemes. While these approaches have delivered remarkable yield gains, they have nearly reached a plateau and are often slow and inefficient when addressing complex traits such as durable disease resistance or climate resilience. The advent of molecular markers, quantitative trait locus (QTL) mapping and marker-assisted selection marked an important transition towards more knowledge-based breeding. Nevertheless, early genomics efforts were constrained by the reliance on single reference genomes, which capture only a fraction of the genetic variation present within a species.

Recent technological advances, particularly in high-throughput short- and long-read sequencing, have transformed plant genetics. In parallel, classical map-based cloning remains a central and highly effective approach for isolating resistance genes, especially when combined with modern pangenomic resources and high-resolution sequencing data. Pangenomics has emerged as a powerful framework to describe the full complement of genes and structural variants across multiple individuals of a species. At the same time, functional validation studies employing Cas endonuclease-mediated genome editing were included to illustrate the translation of genomic discoveries into breeding-relevant outcomes. Together, these approaches are reshaping plant breeding into a predictive and design-oriented discipline.

Barley and wheat are used as illustrative examples to demonstrate how genetic resources from landraces and wild relatives, combined with modern genomics, pangenomics, map-based cloning and functional validation, accelerate the development of resilient crop varieties.

MATERIAL AND METHODS

This article specifically reflects the research activities of our working group on Molecular Genetics and Comparative Genomics including long-term partnerships of the informal Molecular Genetics and Comparative Genomics (MGCG) working group at the Julius Kühn-Institute (Germany) with the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben (Germany), the University of Minnesota (USA), the University of Sydney (Australia), King Abdullah University of Science and Technology (KAUST) and La Trobe University (Australia). This review is based on an integrated analysis of published articles and future plans of MGCG, addressing genetic diversity, disease resistance and modern breeding technologies in cereal crops. Studies were selected that employed gene bank evaluations, phenotypic screening under field and controlled conditions, whole-genome resequencing, pangenome construction and functional genomics approaches.

Particular emphasis was given on research utilizing next-generation sequencing platforms, including Illumina short-read sequencing and long-read technologies such as PacBio, as well as on classical and accelerated map-based cloning approaches for the isolation of resistance genes. Mapping populations included F₂, F₄, recombinant inbred lines (RILs), doubled haploid (DH) lines and near-isogenic lines (NILs) developed for major resistance loci in barley and wheat. Pangenomic analyses integrating multiple assemblies and large-scale resequencing datasets were considered. Functional validation studies employing genome editing were included to illustrate the translation of genomic discoveries into breeding-relevant outcomes.

RESULTS AND DISCUSSION

Genetic Diversity as a Basis for Breeding

Genetic diversity is the foundation of crop improvement. Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop globally and historically ranked third in former Yugoslavia. To preserve its genetic resources, a major collection initiative conducted between 1960 and 1980 established a regional gene bank comprising approximately 150 landraces and 80 cultivars. In 2000, a core set of 106 spring barley landraces was evaluated for agronomic performance and resistance to major diseases. Two accessions, MBR530 and MBR532, exhibited resistance to Barley mild mosaic virus (BaMMV), while genotype MBR1012 showed strong resistance to barley leaf rust caused by *Puccinia hordei*. Phenotypic assessments combined with diversity analyses confirmed the high breeding potential of these accessions (Perovic et al., 2001).

In the last decade, these valuable genotypes have been further characterized within the ExpResBar, IdeMoDeResBar 1 and IdeMoDeResBar 2 projects using advanced sequencing technologies, including PacBio long-read sequencing as well as enriched based sequencing RenSeq and MutRenSeq. Genetic analysis of MBR1012 revealed multiple leaf rust resistance loci on chromosome arm 1HS (König et al., 2012), as well as on 2HL and 5HL (unpublished data), highlighting the complex genetic architecture of leaf rust resistance in this accession. In parallel, high-resolution mapping of the resistance locus on chromosome arm 1HS *RphMBR1012*, was performed using three bi-parental populations derived from crosses between the resistant donor line and the cultivars Scarlett (Fazlikhani et al., 2019), and with Morex and Higgins (unpublished data). This approach narrowed the locus to a 318 kb interval. Subsequent pangenome analysis enabled the development of 30 additional markers for fine mapping; however, the candidate region still contained 19 genes. Together, these results indicated that classical map-based cloning of *RphMBR1012* was not feasible. Therefore, EMS mutagenesis was applied to generate loss-of-function mutants, and whole-genome sequencing of the donor line was undertaken to support the identification of the causal resistance gene. Further functional validation of candidate genes using Cas endonuclease-mediated gene editing and virus-induced gene silencing (VIGS), will enable the isolation of the corresponding genetic factor and afterwards the generation of resistance alleles in elite barley cultivars.

In contrast, map-based cloning of *rym15*, in combination with sequencing of donor line, was successful, leading to the identification of a candidate resistance gene that was further supported by pangenome analysis. Functional validation is currently underway using Cas

endonuclease-mediated gene editing, enabling direct assessment of gene function and, subsequently, the generation of a resistance allele in elite barley.

For resistance against Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV), recent gene editing approaches have generated novel alleles of two previously isolated resistance genes, *rym4/5* and *rym1/11* (Hoffie et al., 2021, 2023). These strategies bypass lengthy introgression breeding and enable the rapid development of resistant cultivars, highlighting the importance of resistance gene isolation for efficient breeding within short timeframes (Stein et al., 2005; Yang et al., 2014).

KASP genotyping further revealed that the resistance genes *rym13* and *rym15*, originally described in East Asian germplasm, are also present in Montenegrin landraces such as MBR530 and MBR532, suggesting a possible bi-phyletic origin of these resistance sources. Together, these findings demonstrate how Balkan genetic resources, when combined with pangenomics and gene editing, can contribute to modern barley breeding and sustainable disease management.

In parallel, wild barley (*H. vulgare* subsp. *spontaneum*) represents an even broader reservoir of genetic variation. Whole-genome resequencing of wild barley diversity collections revealed hundreds of millions of single nucleotide polymorphisms and millions of insertions and deletions. Genome-wide association studies identified novel loci controlling disease resistance and morphological traits, demonstrating the untapped potential of wild germplasm for crop improvement (Spanner et al., 2026).

Pangenome GWAS, *k*-mer Statistics and Gene Landing

Beyond classical SNP-based genome-wide association studies (GWAS), pangenome-enabled GWAS approaches now incorporate structural variants, presence-absence polymorphisms and sequence *k*-mers as genetic features. *k*-mer-based association mapping does not rely on a single reference genome and can capture complex variation originating from gene copy number changes, insertions, deletions and highly diverged haplotypes. This is particularly important for resistance gene clusters and other fast-evolving loci that are often poorly represented in reference assemblies.

In our collaborative research, pangenome GWAS and *k*-mer statistics have substantially accelerated the identification of candidate genes underlying disease resistance and agronomic traits in barley and wheat. By associating specific *k*-mers or pangenome-defined haplotypes with phenotypic variation, trait-associated genomic regions can be narrowed down with high resolution, even in structurally complex loci.

The integration of pangenome GWAS with high-quality genome assemblies enables so-called "gene landing", where candidate genes can be directly placed onto multiple reference genomes and pangenome graphs. This allows precise localization of causal genes, discrimination between allelic variants and paralogous copies, and rapid prioritization of functional candidates for validation. Combined with map-based cloning, CRISPR/Cas-mediated genome editing and VIGS, these approaches greatly accelerate genetic dissection of complex traits and the translation of genomic discoveries into breeding-relevant applications.

Resistance Breeding and Marker Development

Barley leaf rust, caused by *Puccinia hordei* Otth., is one of the most important fungal diseases affecting barley worldwide. The deployment of novel resistance genes is considered the most economical and environmentally sustainable strategy for disease control. Two seedling resistance genes, *RphMBR1012* and *Rph4*, were previously mapped to the distal region of chromosome arm 1HS (König et al., 2012), but their genetic relationship remained unclear for many years.

To resolve this, allelism tests were conducted using F₂ and advanced F₄ populations derived from a cross between MBR1012 and the cultivar Gold. Due to the absence of a *P. hordei* isolate avirulent to both genes in Germany, experiments were performed independently at the University of Minnesota (USA) and the University of Sydney (Australia). F₂ plants and F₄ lines were tested with the isolate AUS220, which is avirulent on both *RphMBR1012* and *Rph4*. Although not yet published, evidence indicating that these two resistance genes are independent is highly relevant for breeding, as it enables their strategic combination to enhance the durability of resistance.

In addition to classical genetic analysis, pangenome-assisted approaches have enabled the development of breeder-friendly molecular markers. A reliable, co-dominant KASP marker (*Rph7_PG1_3*) was developed for the cloned barley leaf rust resistance gene *Rph7* on chromosome 3H. This predictive marker allows efficient pyramiding of *Rph7* with other resistance genes, significantly accelerating selection in breeding programs. Future research aims to identify the origin, prevalence and specific variants of the *Rph7* resistance haplotype using global gene bank genomic datasets.

Genome Editing Based Validation of Virus Resistance Genes

In parallel to classical genetics and pangenomics, our working group applies Cas endonuclease-mediated genome editing to functionally validate resistance mechanisms and generate novel resistance alleles. For instance, two major susceptibility genes for bymovirus infection in barley, *PDIL5-1* and *EIF4E*, were subjected to targeted mutagenesis.

Targeted knockout of *PDIL5-1* resulted in broad resistance to Barley mild mosaic virus (BaMMV) without major negative effects on plant development. Similarly, knockout mutations in *EIF4E* generated homozygous mutant plants that were fully resistant to BaMMV following mechanical inoculation under controlled growth chamber and glasshouse conditions. The mutants showed normal vegetative growth and grain formation, although a reduction in total grain number per plant was observed. These findings are based on two major studies conducted by our group, demonstrating that targeted knockout of the susceptibility genes *PDIL5-1* or *EIF4E* confers resistance to bymoviruses in barley (Hoffie et al., 2021, 2023). Currently, barley lines with new *EIF4E* alleles generated by base editing are under testing by BaMMV infection assays.

All genome editing-derived mutants were rigorously tested under controlled environmental conditions, including growth chambers and glasshouses, using mechanical virus inoculation and serological assays (DAS-ELISA). These experiments confirmed stable resistance phenotypes and enabled detailed phenotypic characterization. However, large-

scale multi-location field trials of the edited lines have not yet been completed, as current European Union regulations restrict the release of genome-edited crops into open-field environments. Large-scale multi-location field trials are planned once European Union regulations for genome-edited crops are adapted to allow open-field testing.

Map-Based Cloning of Resistance Genes

Map-based cloning represents a core research strategy of our working group for the isolation of resistance genes against soil-borne viruses and fungal pathogens in barley and wheat. Using high-resolution genetic mapping, dense marker saturation, pangenome-informed candidate gene discovery and long-read sequencing, we are currently isolating several key resistance loci, including the soil-borne virus resistance genes *rym13* and *rym15*, as well as the barley leaf rust resistance gene *RphMBR1012*. Resistance sources were identified through systematic screening with German isolates of soil-borne viruses and rust, ensuring the relevance of mapped resistance loci for Central European pathogen populations.

The integration of multiple reference-quality genome assemblies and barley pangenome resources has substantially accelerated this process by enabling precise localization of resistance loci within structurally complex genomic regions rich in copy number and presence–absence variation. Functional validation is performed using CRISPR/Cas-mediated genome editing allowing rapid confirmation of candidate gene function.

Overall, the map-based cloning pipeline in our group combines (i) development of appropriate mapping populations, (ii) high-resolution phenotyping, (iii) dense genotyping using pangenome-derived markers, (iv) candidate gene identification from multiple genome assemblies, and (v) functional validation. This integrated approach ensures efficient translation of genetic discoveries into breeding-relevant outcomes.

Comparative Insights from Wheat

Bread wheat (*Triticum aestivum*) provides complementary insights into the value of pangenomics and wild relatives. Sequencing and analysis of multiple *Aegilops tauschii* genomes clarified the complex origin of the wheat D genome and enabled cloning of resistance genes from highly duplicated and structurally complex loci. These studies highlighted the importance of broad sampling and pangenomic approaches, particularly in polyploid crops where gene redundancy complicates genetic analyses.

Pangenome-Guided Resistance Gene Discovery in Wheat

Recent large-scale pangenomic studies on the origin and evolution of the bread wheat D genome (Cavalet-Giorsa et al., 2024) have demonstrated the immense value of wild relatives, particularly *Aegilops tauschii*, as reservoirs of untapped genetic diversity. By generating high-quality genome assemblies representing multiple *Ae. tauschii* lineages and combining them with *k*-mer-based GWAS, researchers were able to clone disease resistance genes and resolve complex resistance loci by distinguishing alleles from paralogous gene copies. These resources revealed that the wheat D genome is a mosaic derived from multiple geographically distinct *Ae. tauschii* subpopulations, highlighting the evolutionary and functional complexity of resistance loci and the importance of haplotype-level resolution for gene discovery.

Inspired by these advances, our working group is currently applying pangenome-based strategies for the isolation of resistance genes in wheat. We are screening diverse wheat germplasm with German isolates of soil-borne viruses, rusts, and powdery mildew to identify novel resistance sources. Using pangenome GWAS, *k*-mer statistics and high-resolution genetic mapping, we aim to rapidly localize candidate genes in structurally complex genomic regions. Subsequent functional validation will enable precise characterization of resistance mechanisms and their deployment in breeding programs.

CONCLUSION

This conference contribution highlights the integrated research strategy of our working group, combining gene bank resources, pangenomics, map-based cloning and genome editing to accelerate resistance breeding in barley and wheat.

The integration of pangenomics and gene editing marks a paradigm shift in plant genetics and breeding. By capturing the full spectrum of genetic variation and enabling precise allele deployment, these technologies provide powerful tools to address challenges posed by climate change, emerging diseases and sustainability demands. Barley and wheat research demonstrates how gene bank resources, advanced genomics and genome editing can be combined to accelerate the development of resilient crop varieties. Continued expansion of pangenome resources and responsible implementation of gene editing will be essential for securing future food production. These integrated approaches position our working group and its international collaborators at the forefront of modern resistance breeding in cereals.

REFERENCES

- Cavalet-Giorsa, E., González-Muñoz, A., Dragan, P. *et al.* (2024). Origin and evolution of the bread wheat D genome. *Nature*, 633, 848-855. doi: 10.1038/s41586-024-07808-z.
- Fazlikhani, L., Kopahnke, D., Keilwagen, J., Deising, H.B., Ordon, F., Perovic, D. (2019). High resolution mapping of RphMBR1012 conferring resistance to *Puccinia hordei* in barley (*Hordeum vulgare* L.). *Frontiers in Plant Science*, 10, Art. 640. doi: 10.3389/fpls.2019.00640
- Hoffie, R.E., Perovic, D., Habekuß, A., Ordon, F., Kumlehn, J. (2023). Novel resistance to the Bymovirus BaMMV established by targeted mutagenesis of the PDIL5-1 susceptibility gene in barley. *Plant Biotechnology Journal*, 1-11. doi: 10.1111/pbi.13948.
- Hoffie, R.E., Otto, I., Perovic, D., Budhagatapalli, N., Habekuß, A., Ordon, F., Kumlehn, J. (2021). Targeted Knockout of Eukaryotic Translation Initiation Factor 4E Confers Bymovirus Resistance in Winter Barley. *Frontiers in Genome Editing* 3, Article 784233. doi: 10.3389/fgeed.2021.784233.
- Jayakodi, M., Lu, Q., Pidon, H., Perovic, D. *et al.* (2024). Structural variation in the pangenome of wild and domesticated barley. *Nature*, 636, 654-662, doi: 10.1038/s41586-024-08187-1
- König, J., Steffenson, B., Kopahnke, D., Pržulj, N., Romeis, T., Ordon, F., Perović, D. (2012). Genetic mapping of novel leaf rust (*Puccinia hordei* Oth) resistance in barley landrace MBR1012. *Molecular breeding*, 30, 1253-1264. doi: 10.1007/s11032-012-9712-0
- Perovic, D., Przulj, N., Milovanovic, M., Prodanovic, S., Perovic, J., Kopahnke, D., Ordon, F., Graner, A. (2001). Characterisation of spring barley genetic resources in Yugoslavia. *Rudolf Mansfeld and plant genetic resources. Schriften zu Genetischen Ressourcen, Band 22, 301-306, Gatersleben, Germany.*
- Spanner, R., Sallam, A.H., Ordon, F., ...Perovic, D. *et al.* (2026). Whole-genome resequencing of the wild barley diversity collection: a resource for identifying and exploiting genetic variation for cultivated barley improvement. *G3*. jkaf261. doi: 10.1093/g3journal/jkaf261
- Stein, N., Perovic, D., Kumlehn, J., Pellio, B., Stracke, S., Streng, S., Ordon, F., Graner, A. (2005). The eukaryotic translation initiation factor 4E confers multiallelic recessive Bymovirus resistance in *Hordeum vulgare* (L.). *Plant J.*, 42, 912-922.
- Yang, P., Lüpken, T., Habekuß, A., Hensel, G., Steuernagel, B., Kilian, B. *et al.* (2014). Protein disulfide isomerase like 5-1 is a susceptibility factor to plant viruses. *Proc. Natl. Acad. Sci.*, 111, 2104-2109. doi: 10.1073/pnas.1320362111
- Wiles, D., Gurung, K., Tongson, E., Park, R., Cai, Y., Viradia, B., Okuda, K.S., Perovic, D., Dracatos, P.M. (2025). Pangenome-assisted development and validation of a predictive KASP marker for the barley leaf rust resistance gene Rph7. *Frontiers in Agronomy*, 7, 1670733. doi: 10.3389/fagro.2025.1670733