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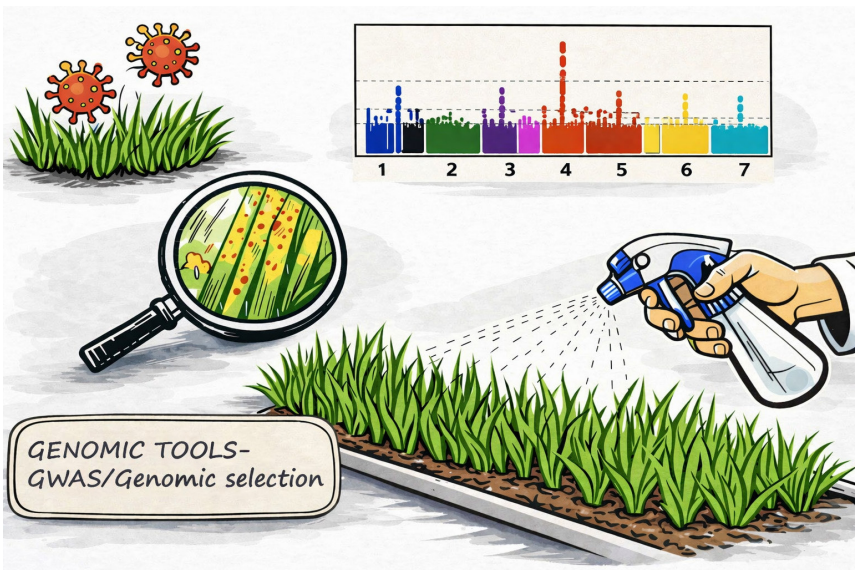

# Genome-wide analysis of quantitative disease resistance in spring barley

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# Genome-wide analysis of quantitative disease resistance in spring barley


## Abstract

Fungal diseases represent a major challenge in spring barley production, affecting both yield and quality. Resistance to these pathogens is complex and quantitatively inherited, making it difficult to perform genetic dissection and selection. This study evaluated quantitative resistance to scald, powdery mildew, and net form net blotch (NFNB) using genome-wide approaches in diverse spring barley panels.

Genome-wide association study (GWAS) analyses identified several quantitative trait loci (QTL) associated with resistance to the targeted diseases, including both previously reported and novel loci. The number of detected QTL and their effects varied among diseases and populations, indicating the strong influence of genetic background on resistance architecture.

Population composition and genetic relatedness between the training and validation sets influenced genomic prediction accuracy. When these sets were closely related, marker effects were estimated more consistently, whereas prediction accuracy decreased when these populations were genetically distant. Although increasing the training population size improved prediction accuracy in some scenarios, the predictions were inconsistent and depended on the underlying genetic structure. Validation at different stages indicated limited transferability of genomic signals from seedling-stage to adult-plant resistance in field conditions.

These results indicate that both population composition and phenotyping context are key factors for detecting resistance loci and the performance of genomic prediction models. The results emphasise the need to account for genetic background and crop growth stage when applying genome-wide approaches to breeding for disease resistance in spring barley.



Keywords: barley, AUDPC, wMAS, GWAS, genomic prediction, scald, powdery mildew, net form net blotch, rrBLUP

# Genomomfattande analys av kvantitativ sjukdomsresistens hos vårkorn


## Abstract

Svampsjukdomar är en stor utmaning inom vårkornsproduktion och påverkar både avkastning och kvalitet. Resistens mot dessa patogener är genetiskt komplex och kvantitativt nedärvd, vilket gör det svårt att utföra genetisk analys och selektion inom förädling. Denna studie utvärderade kvantitativ resistens mot sköldfläcksjuka, mjöldagg och nätformig bladfläcksjuka med hjälp av metoder baserade på genetiska markörer fördelade över hela genomet i olika vårkornspaneler.

Genomvida associationsstudier identifierade flera genetiska regioner (QTL) associerade med resistens mot dessa sjukdomar, inklusive både tidigare rapporterade och nya loci. Antalet detekterade QTL och deras effekter varierade beroende på sjukdom och population, vilket tyder på att genetisk bakgrund har ett starkt inflytande på resistensarkitekturen.

Populationssammansättning och genetiskt släktskap mellan tränings- och valideringspopulation påverkar den genomiska prediktionsnoggrannheten. När dessa uppsättningar var nära besläktade uppskattades marköreffekter mer konsekvent, medan prediktionsnoggrannheten minskade när dessa populationer var genetiskt avlägsna. Även om ökning av träningspopulationsstorleken förbättrade prediktionsnoggrannheten i vissa scenarier, var prediktionerna inkonsekventa och berodde på den underliggande genetiska strukturen. Validering i olika växtstadier indikerade en begränsad överförbarhet av prediktionsmodeller från plantstadiet till resistens hos vuxna växter under fältförhållanden.

Dessa resultat visar att både populationssammansättning och fenotypningskontext är nyckelfaktorer för att identifiera resistensloci och för de genomiska prediktionsmodellernas prediktionsförmåga. Resultaten betonar vikten av att ta hänsyn till genetisk bakgrund och grödans tillväxtstadium vid tillämpning genomvida markörbaserade metoder iförädling för sjukdomsresistens hos vårkorn.



Nyckelord: korn, AUDPC, wMAS, GWAS, genomisk prediktion, sköldfläcksjuka, mjöldagg, nätformig bladfläcksjuka, rrBLUP

# Contents

# List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Noe, S. M., Åstrand, J., Zakieh, M., Singh, P. K., Johansson, E., & Chawade, A. (2025). Harnessing novel genetic markers for scald resistance from gene bank spring barley genotypes. *BMC Plant Biology*, *25*(1), 781. https://doi.org/10.1186/s12870-025-06813-2

II. Noe, S. M., Singh, P. K., Odilbekov, F., Johansson, E., & Chawade, A. Predicting net form net blotch resistance across diverse barley panels. (Submitted)

III. Noe, S. M., Singh, P. K., Odilbekov, F., Johansson, E., & Chawade, A. (2025). Comparing wMAS, GWAS, and genomic prediction for selecting powdery mildew-resistant spring barley genotypes. *BMC Genomics*, *26*(1), 1091. https://doi.org/10.1186/s12864-025-12395-y

All published papers are published open access.

The contribution of Su Myat Noe to the papers included in this thesis was as follows:

I. Designed the experiments together with co-authors. Conducted greenhouse experiments in Biotron, Alnarp. Evaluated scald disease resistance at seedling stage. Analysed data and wrote the manuscript with input from co-authors.

II. Designed the experiments together with co-authors. Conducted greenhouse experiments in Lantmännen Lantbruk AB, Svalöv, and evaluated powdery mildew disease resistance at seedling stage. Analysed data and wrote the manuscript with input from co-authors.

III. Designed the experiments together with co-authors. Conducted greenhouse experiments in Lantmännen Lantbruk AB, Svalöv. Evaluated net form net blotch disease resistance at seedling stage. Analysed data and wrote the manuscript with input from co-authors.

# List of figures

# Abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| APR | Adult-plant resistance |
| AUDPC | Area under disease progress curve |
| BLINK | Bayesian-information and linkage-disequilibrium iteratively nested keyway |
| BLUE | Best linear unbiased estimation |
| BLUP | Best linear unbiased prediction |
| Chr. | Chromosome |
| CV | Cross-validation |
| DPI | Days post inoculation |
| Effect | Allelic effect estimate |
| FDR | False discovery rate |
| GEBV | Genomic estimated breeding values |
| GP | Genomic prediction |
| GS | Genomic selection |
| GWAS | Genome-wide association studies |
| GWAS_FIX_rrBLUP | GWAS-incorporated genomic prediction |
| $H^2$ | Broad-sensed heritability |
| He_train | Expected heterozygosity (training population) |
| HSD | Honestly significant difference (Tukey's HSD test) |
| LD | Linkage disequilibrium |
| LD half-decay | Distance where LD declines to half of its maximum value |
| LD_train/LD_valid | LD decay in training/validation population |
| LSD | Least significant difference |
| MAF | Minor allele frequency |
| Mbp | Megabase pair |
| Me | Effective number of chromosome segments |
| MTA | Marker–trait association |
| NFNB | Net form net blotch |
| PA | Predictive ability |
| Pos (Mbp) | Physical position (megabase pair) |
| PS | Phenotypic selection |

| | |
|---|---|
| PVE (%) | Phenotypic variance explained |
| QTL | Quantitative trait loci |
| SFNB | Spot form net blotch |
| SNP | Single nucleotide polymorphism |
| SS | Seedling-stage resistance |
| STD_rrBLUP | Standard GP |
| TS | Training set |
| VS | Validation set |
| wMAS | Weighted marker-assisted selection |

# 1. Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereal crops globally. In 2023, approximately 146 million tons were produced worldwide, with more than half of the total production originating in Europe (FAOSTAT 2025) (Figure 1). Barley contributes approximately 30% of the total grain production in Sweden with the majority of production occurring in the southern part of the country (Parsons 2025). The most commonly cultivated type of barley in Sweden is two-row spring barley (Lantmännen 2023).

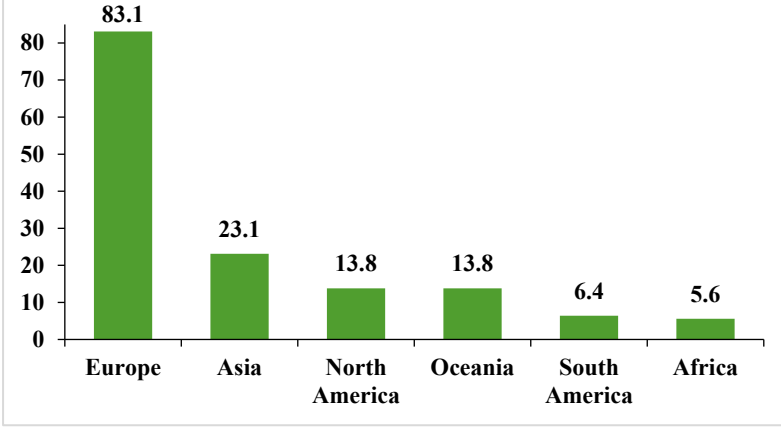


*Figure 1*. Global barley production in 2023 (FAOSTAT).

Fungal diseases such as scald, net blotch, stem rust, leaf rust, and powdery mildew significantly reduce grain yield and quality, and affect the biomass harvested for feed (Yu et al. 2021). To try and prevent such diseases, fungicide application and the cultivation of resistant cultivars, either individually or as part of an integrated approach, are widely used as control measures (Taylor & Cunniffe 2023). To reduce reliance on fungicide whilst maintaining stable yields, a greater emphasis has been placed on developing cultivars with strong and durable resistance.

Resistance to various barley diseases is quantitatively inherited and controlled by multiple loci, each contributing a small proportion of the phenotypic variation (Brown 2021). This polygenic architecture limits the efficiency of traditional marker-assisted selection which is based on biparental population mapping and is better suited for major-effect loci (Shahi et al. 2022). Genome-wide approaches, including genome-wide

association studies (GWAS) and genomic prediction (GP), provide alternatives for dissecting and utilising quantitative resistance. GWAS identify genomic regions associated with disease resistance, whereas GP estimates breeding values using genome-wide marker information (Li et al. 2021; Zhang et al. 2023; Alemu et al. 2024).

Despite these advances, the effective application of these approaches across the broad genetic diversity of spring barley, including both foundational germplasm and advanced breeding lines, remains limited. Modern crop improvement has largely relied on elite breeding lines, which possess a narrow genetic diversity, whilst genebank collections preserve broader allelic variation that is often absent in advanced breeding materials (Yuan et al. 2025). Integrating diverse germplasm with elite lines offers opportunities to identify novel alleles and evaluate their breeding potential (Yuan et al. 2024). Such a combination is particularly valuable for genome-wide analyses, where both locus detection and prediction performance depend on the underlying genetic diversity and population composition.

In addition, disease resistance can vary across crop growth stages and environments. Resistance observed at the seedling stage may not fully represent adult-plant resistance, and similarly, environmental variability and genotype-by-environment interactions contribute substantially to phenotypic outcomes (Crossa et al. 2017; Juliana et al. 2017). Thus, understanding how genomic signals transfer across populations, environments, and developmental stages is essential for the effective implementation of genome-wide strategies in breeding programmes (Hickey et al. 2017).

This thesis adopts a comparative and population-specific genomic framework across major foliar diseases of spring barley. The individual studies collectively investigate quantitative resistance across diverse genetic materials and evaluate the performance and transferability of genome-wide approaches under different population compositions and phenotyping contexts.

# 2. Background

## 2.1 Barley: one of the first crops to be domesticated by humans

Barley is classified under the genus Hordeum. This genus includes 32 other species that share similar morphology such as bearing triplet spikelets at each rachis node. Hordeum is largely distributed in temperate regions, with its origins in the Mediterranean, Central Asia, and parts of the Americas (Bothmer et al. 1995). Wild barley (*Hordeum spontaneum*) is the ancestor of cultivated barley, including both two-row and six-row barley. This difference in row type is due to mutations that occurred during domestication (Bothmer et al. 1995). Important traits for domestication include rachis brittleness, dormancy, growth habit, and resistance to abiotic and biotic stresses (Dai & Zhang 2016). Barley contains a high nutritional value with a moderate protein content, high carbohydrate levels, and dietary fibre, such as β-glucan which is known to lower LDL cholesterol (Keenan et al. 2007). Globally, around 400,000 barley accessions are conserved and the majority of these collections are maintained at international and national genebanks, including ICARDA and various national repositories across Asia, the Americas, and Europe (ICARDA 2016).

## 2.2 Genebank germplasm: a genetic resource for developing resistance cultivars

Germplasms represent important sources for crop improvement as they maintain allelic diversity that is often absent in modern breeding lines (Kearsey 1997). Worldwide genebanks conserve landraces and wild relatives that harbour resistant alleles lost during evolutionary or selection processes (CGIAR 2019; Reynolds & Braun 2022). Advanced genotyping technologies enable these resources to be explored more efficiently and increase the chance of detecting more causal variants associated with different diseases (Rangan et al. 2023).

For example, GWAS based on diverse wheat collections have revealed new loci associated with fusarium head blight resistance, demonstrating the value of broad genetic diversity in trait discovery (Zakieh et al. 2021). One study which genotyped 812 barley plant genetic resources, together with elite lines,

using whole-genome sequencing revealed substantial genetic and phenotypic variation for agronomic traits including scald resistance, and provided a high-resolution data resource useful for future barley breeding (Yuan et al. 2024).

Moreover, GP studies using genebank materials also demonstrated that marker data from diverse panels can improve prediction accuracy for complex traits. For example, a study conducted to test seedling-stage Septoria tritici blotch (STB) resistance using winter wheat germplasm from the Nordic region identified QTL via GWAS and integrated them into a genomic prediction model, achieving predictive ability exceeding 0.60 (Odilbekov et al. 2019). Another study with a similar statistical approach was performed to examine adult-plant resistance to STB and powdery mildew and reported predictive abilities ranging from 53-75% across multiple environments (Alemu et al. 2021). These results underscore the value of genebank germplasm for detecting novel resistance alleles and demonstrate the advantage of genomic tools for exploring these genetic resources for disease resistance in wheat.

Beyond disease resistance, genebank materials have also been used to identify loci associated with agronomic traits such as grain quality and abiotic stress resistance, indicating their value for multiple breeding targets. For instance, genomic analyses in cereals including sorghum, rice, and maize have identified QTL associated with yield-related and other complex traits (Wu et al. 2021; Min et al. 2025; Naing et al. 2025).

## 2.3 Quantitative disease resistance and genetic architecture

Quantitative resistance is typically controlled by multiple loci with small to moderate effects, rather than a single major resistance gene (Chauveau & Roby 2024). This type of resistance is often more durable, as it is less likely to be overcome by pathogen evolution (Camenzind et al. 2024; Yuan et al. 2024). The quantitative resistance is complex and affected by environmental conditions, making it difficult to dissect and predict (Pati et al. 2025). Genome-wide approaches, such as GWAS and genomic selection (GS), are therefore widely used to capture both major and minor-effect loci that impact resistance.

### 2.3.1 *Rhynchosporium graminicola*: a causal pathogen of scald (Paper I)

The causal pathogen of scald disease was initially considered to be *Rhynchosporium secalis,* which infects rye, triticale, and barley. However, studies later found that the pathogen is host-specific, and the strain infecting barley is a distinct species known as *Rhynchosporium commune* (Zaffarano et al. 2011). Recent studies have suggested that the species should be referred to as *Rhynchosporium graminicola (R. graminicola)* due to nomenclatural priority (Crous et al. 2021). It primarily infects barley, with limited pathogenicity reported on closely related grasses (Crous et al. 2021). *R. graminicola* is a hemibiotrophic pathogen that parasitises the host by invading living tissues in a biotrophic manner and later killing the host cells during the necrotrophic phase (Divon and Fluhr 2007). Although it is predominantly known to reproduce asexually, recent studies indicate that sexual reproduction may also occur, despite the fact that ascocarps (sexual fruiting bodies) have not been observed (Zhang et al. 2020). Crop residues, seeds, and soil serve as the primary sources of inoculum, whilst rain splashes help to disperse conidia to neighbouring plants (Zhang et al. 2020). Scald disease develops under cool and moist conditions, particularly at temperatures between 12-20 °C, with frequent rainfall and high humidity favouring disease spread (Carmona et al. 1997).

After infection occurs, conidia germinate on the leaf surface, penetrate the host cuticle, and progressively colonise the leaf tissues, resulting in cell collapse and lesion formation (Ayesu-Offei & Clare 1970; Zhan et al. 2008). At an early stage of infection, greyish, water-soaked lesions appear on the leaf surface, and these gradually develop into chlorotic and necrotic lesions surrounded by a deep-brown margin on the leaf blade (Figure 2) (Lehnackers & Knogge 1990; Jackson 1997).

*Figure 2.* Scald *(Rhynchosporium graminicola)* infection - Photo: Su Myat Noe.

Scald disease can also develop without visible symptoms whilst the fungus grows inside the host tissues, allowing the pathogen to be transmitted to harvested seeds . These infected seeds serve as an important source of inoculum across generations and long-distance transmission across seasons (Fountaine et al. 2010; Stefansson et al. 2012). Yield loss due to scald ranges from 10-40%, but may reach 100% in fields lacking crop rotation in several countries across Asia, Africa, and South America (Bouajila et al. 2007; Beigi et al. 2013; Cope et al. 2021).

### 2.3.2 *Pyrenophora teres* f. *teres*: the pathogen behind net blotch (Paper II)

The causal pathogen of net blotch in barley, *Pyrenophora teres* (anamorph *Drechslera teres*), is one of the most threatening pathogens to barley production. It occurs in two forms: *P. teres* f. *teres*, which causes net form net blotch (NFNB), and *P. teres* f. *maculata*, which causes spot-form net blotch (SFNB) (Smedegard-Petersen 1971; Backes et al. 2021).
Similar to *R. graminicola*, *P. teres* f. *teres* (*Ptt*) survives on crop residues and wild grass hosts, which serve as primary inoculum sources. Spore production favours cool to moderately warm temperatures and high humidity. The pathogen reproduces sexually on overwintered debris and asexually during the vegetative stage of the crop (Piening 1968; Duczek et al. 1999).

*Figure 3.* Net form net blotch (*Pyrenophora teres* f. *teres*) infection - Photo: Su Myat Noe.

Infection begins when spores land on the leaf surface, germinate, and later penetrate the epidermal cells. After colonisation, dark brown, net-like lesions develop and spread across the leaf blade, reducing photosynthetic capacity (Figure 3). Young plants are the most susceptible, as their cuticles are still thin which consequently facilitates pathogen penetration (Khan & Boyd 1969; Douiyssi et al. 1998).

Net blotch has been reported in several barley-growing regions for decades (Yousfi & Ezzahiri 2002; Akhavan et al. 2016). Yield losses due to NFNB reach 40% and severe epidemics can occur under favourable environmental conditions, with reports of near-complete crop losses. Furthermore, NFNB negatively affects grain plumpness and kernel size, both of which are important quality traits for malting barley (Backes et al. 2021).

*Ptt* populations are genetically diverse and capable of sexual recombination, increasing their likelihood of overcoming host plant resistance and fungicide resistance over time (Jalli 2011; Poudel et al. 2019). The combination of favourable climate conditions and widespread inoculum sources in barley production renders net blotch a persistent challenge and thus it remains a key priority for resistance breeding.

### 2.3.3 *Blumeria hordei*: the causal pathogen of powdery mildew (Paper III)

Powdery mildew is an obligate biotroph that infects Triticeae, including wheat, rye, and barley (Liu et al. 2021). The pathogen is host-specific, and the one that infects barley is *Blumeria hordei* (*Bh*).

Infection commences within 24 hours after airborne conidia has landed on the leaf surface and germination is followed by penetration of the epidermal cell through an appressorium (Carver 1996). Once inside the host cell, the fungus establishes a haustorium which extracts nutrients whilst keeping the host cell alive and metabolically active.

As the fungal growth continues to occur on the leaf surface, epiphytic mycelium develops and subsequently produces conidiophores that generate chains of conidia that are easily dispersed by the wind. Typical symptoms appear as white to greyish powdery patches on the leaf surface after infection (Figure 4), followed by leaf yellowing and reduced photosynthetic efficiency (Hacquard 2014).

Given that *Bh* is an obligate biotroph, its entire life cycle depends on living host tissue, and it cannot be cultured on artificial media (Hacquard 2014). Continuous availability of green plant material, in areas where barley is grown year-round, predominantly maintains its asexual life cycle. Sexual reproduction may also occur when compatible isolates meet, usually at the end of the growing season (Wolfe & McDermott 1994).

*Figure 4.* Powdery mildew (*Blumeria hordei*) infection - Photo: Su Myat Noe.

Powdery mildew can survive at moderate temperatures (15-22 °C) and high humidity. Unlike other foliar pathogens, *Bh* does not require free water, and prolonged leaf wetness can even inhibit spore germination (Timmerman & Wegulo 2010). Favourable conditions for powdery mildew include humid but not wet canopies, reduced sunlight, warm temperatures, and dense or shaded cropping conditions that limit proper air circulation.

Yield loss ranges from 10-30%, however, reports of 50% yield reductions have been made when susceptible cultivars are grown in disease-prone areas. Severe infections can also affect grain size and malting quality, demonstrating the importance of powdery mildew in barley breeding production (DPIRD 2024).

To effectively control powdery mildew, a combination of measures is needed rather than an individual approach. Growing resistant cultivars is one of the most sustainable strategies and integrating it with agronomic practices such as crop rotation with non-cereal species, moderate use of nitrogen fertiliser, and appropriate seed rates may reduce disease pressure to a certain extent. Fungicides with a recommended dosage may also be applied, when necessary, as a part of an integrated approach that decreases the reliance on any single control method.

## 2.4 Host resistance to scald, NFNB, and powdery mildew

As is the case for other crops, disease resistance in cereals can arise from different types of genetic control, ranging from single genes with major effects to complex polygenic backgrounds. Major genes (R genes) provide race-specific resistance; however, this type of resistance is often short-term, as pathogens can rapidly evolve to overcome R genes and break down the host's resistance mechanisms (Barua et al. 1993).

To date, eleven major genes have been mapped for scald resistance (Abbott et al. 1991; 1995; Graner & Tekauz 1996; Garvin et al. 2000; Genger et al. 2003; Patil et al. 2003; Pickering et al. 2006; Wagner et al. 2008; Coulter et al. 2019). Among them, *Rrs1* was the earliest to be identified in barley and has been mapped to chromosome 3H (Graner & Tekauz 1996).

Although NFNB resistance has been considered predominantly quantitative, a recent study has validated *Rpt5* as a major resistant gene against NFNB. It encodes a receptor-like protein (RLP) that provides dominant resistance to *Ptt* (Effertz et al. 2024). The *Rpt5* locus appears to be complex, as a dominant resistance gene (*Rpt5*) and a dominant susceptibility allele (*Spt1*) are located within the same locus. Different *Ptt* isolates carry different effectors, and their interaction with the *Rpt5*/*Spt1* locus determines whether resistance or susceptibility is expressed (Koladia et al. 2017).

Similarly, more than 30 allelic variants along with several major genes, including *Mla1, Mla6, Mlg, and Mlh,* were identified for powdery mildew resistance (Czembor & Czembor 2021). These genes encode NLR immune receptors that recognise AVR effectors of specific *Bh* isolates and activate host defence such as hypersensitive cell death (Velásquez-Zapata et al. 2023). However, since the *Bh* population is genetically diverse and coevolves rapidly, it can overcome MLA-mediated resistance through amino-acid substitution mutations (Lu et al. 2016; Saur et al. 2019). It exhibits a common limitation of race-specific R genes that are highly effective but not durable.

Unlike major gene resistance, quantitative resistance tends to be more durable because multiple loci control it, each contributing a small to moderate effect that comes from a broader genetic background. Several QTL associated with scald, NFNB, and powdery mildew resistance have been identified and applied in marker-assisted breeding programmes. These loci are distributed across the barley genome whilst the majority of NFNB-resistant QTL are located on chromosome 6H (Richards et al. 2024). In contrast, QTL associated with scald (frequent on chromosome 3H) and powdery mildew spread more widely across all barley chromosomes depending on the germplasm used (Gupta et al. 2018; Novakazi et al. 2020; Zhang et al. 2020; Ge et al. 2021). However, quantitative resistance can also erode over time, as pathogens can still adapt through various mechanisms, including multiple minor mutations, direct and indirect pathogen adaptation, and changes in the host's genetic background which arise from unfavourable allele combinations (Pilet-Nayel et al. 2017).

## 2.5 Genomic tools for disease resistance breeding

Disease resistance breeding initially relied on phenotypic selection, a conventional approach based on a visual assessment of genotypes with desirable traits, and later evolved into marker-assisted selection (MAS) which uses DNA markers linked to the targeted gene or QTL (Hasan et al. 2021). Earlier molecular breeding approaches were based on QTL mapping in biparental populations, however, low mapping resolution and narrow allelic diversity that comes from only two parents' variation limited its application. In addition, many disease resistance traits are quantitatively

inherited, and often influenced by genotype-by-environment interactions (Liu et al. 2021; Purkaystha et al. 2024).

Advanced genomic technologies shifted resistance breeding toward association mapping approaches by exploring the existing but previously untouched genetic diversity (Novakazi et al. 2019). GWAS combine phenotypic data and genome-wide markers to detect disease resistance loci at a higher resolution than QTL mapping. GWAS successfully identified resistance loci for several diseases that infect barley as well as other crops (Zakieh et al. 2021). Moreover, a GWAS study that evaluated more than 600 barley accessions for powdery mildew resistance at both seedling and adult-plant stages identified two major QTL. One QTL was associated with adult-plant resistance, whilst the other with seedling-stage resistance (Guo et al. 2024). Additionally, an association mapping study using a mixed-origin barley panel identified 19 genetic markers associated with scald resistance, eight of which were novel (Ijaz et al. 2024). A barley panel was analysed for NFNB resistance, and of the 54 identified QTL, 16 were novel and three were shared between the seedling and adult-plant stages (Rehman et al. 2025). These findings demonstrate the value of GWAS in identifying novel genetic regions to facilitate marker-assisted breeding for disease-resistant barley.

However, whilst GWAS improves mapping resolution compared to QTL mapping, it also possesses certain limitations, including the need for large sample sizes and high marker density, as well as limited statistical power to detect small-effect loci contributing to missing heritability, i.e., genetic variance not explained by significant SNPs (single nucleotide polymorphisms). Missing heritability may result from incomplete linkage, rare variants, or insufficient sample size (Korte & Farlow 2013; Ibrahim et al. 2020; Khan et al. 2021).

Genomic selection addresses these constraints by incorporating genome-wide markers into a single model instead of relying solely on the significance of individual markers (Zhang et al. 2023; Alemu et al. 2024). GS effectively captures a larger proportion of genetic variance by considering both small and large-effect loci, making it particularly effective for complex traits such as disease resistance (Alemu et al. 2024; Kumari et al. 2024). A GS study that evaluated over 1,000 barley genotypes for scald and powdery mildew resistance achieved moderate to high predictive ability. This result highlights the potential of GS and the barley genotypes for developing resistant

varieties (Yuan et al. 2024). In another study, the resistance of 361 elite wheat lines to five fungal diseases was predicted through the application of 19 genomic prediction approaches. This study indicated that RR-BLUP consistently demonstrated the highest predictive accuracy, and identified the most resistant genotypes for powdery mildew, stripe rust, fusarium head blight, and STB (Heilmann et al. 2025). Additionally, a recent study evaluated eight machine learning methods to predict three rice diseases (rice blast, black-streaked dwarf virus, and sheath blight) and two wheat diseases (wheat blast and stripe rust), and three models achieved a prediction accuracy higher than 85% (Liu et al. 2024). Further, one study evaluated the performance of sorghum F1 hybrids for yield and yield components using a GP approach and reported that prediction accuracy improved as the training population size increased (Maulana et al. 2023).

The integration of GWAS and GS represents a promising approach, as loci identified through GWAS can be incorporated into GP models to enhance prediction accuracy (Odilbekov et al. 2019; Tsai et al. 2020; Zakieh et al. 2023; Meuwissen et al. 2024; Kumaran et al. 2025). Another recent study utilising a spring wheat panel evaluated seven GP models for yield and yield component traits, and reported improved predictive abilities ranging from 7.2% to 22.5% (Gao et al. 2025). This combined approach allows major resistance genes alongside polygenic background effects to be assessed, supporting a more durable and broad-spectrum resistance (Spindel et al. 2016). Despite their increasing application, the effectiveness of GWAS and GS can still vary depending on trait complexity, phenotypic data quality, and population composition, highlighting the need to evaluate these tools across genetically diverse barley populations (Alemu et al. 2024).

# 3. Objectives

The overall objective of this Ph.D. research is to investigate quantitative disease resistance in spring barley using genome-wide approaches across diverse genotype panels. By identifying resistance loci, population-level genetic characterisation, and genomic prediction analyses, this research aims to enhance knowledge about resistance architecture and support the development of barley cultivars with more durable resistance to major foliar diseases, including scald, net form net blotch, and powdery mildew. Based on this overall objective, the specific objectives of Papers I-III were to:

- Identify QTL associated with resistance to scald, NFNB, and powdery mildew through GWAS (Papers I-III).
- Compare newly detected loci with previously reported regions to assess their novelty and potential relevance for barley breeding (Papers I-III).
- Characterise genetic diversity, population structure, and relatedness within the barley panels to evaluate how allelic composition influences resistance patterns (Papers I & III).
- Develop and evaluate GP models for NFNB and powdery mildew diseases under different training populations, marker sets, and cross-validation schemes (Papers II & III).
- Compare different selection strategies to assess their effectiveness in identifying superior breeding lines for powdery mildew resistance (Paper III).

# 4. Materials and methods

The experimental conditions and phenotyping protocols differed among diseases; however, downstream data processing and association analyses followed a largely consistent analytical framework, with disease-specific extensions applied where appropriate.

## 4.1 Plant material

The spring barley used in this thesis originated from two main sources: (i) genebank accessions obtained from the Nordic Genetic Resource Centre (NordGen), Sweden, and (ii) advanced breeding lines provided by Lantmännen AB, Svalöv, Sweden. The majority of the genebank material consisted of Nordic landraces and was used to study resistance to scald and NFNB, whilst the breeding materials, which were both two-row and six-row types, were used to evaluate resistance to powdery mildew and NFNB. All materials were evaluated for the above-mentioned foliar disease at the seedling stage under controlled greenhouse conditions. For NFNB, a subset of genotypes was additionally evaluated at the adult-plant stage under field conditions for cross-stage validation purposes. The number of genotypes and experimental designs varied among papers due to differences in experimental facilities.

### 4.1.1 Study populations and genetic structure

The population structure of the study populations was evaluated using principal component analysis (PCA) based on genome-wide markers. PCA was conducted in the GAPIT 3 package, and the first three principal components were used to visualise genetic relationships among the six-row (breeding), two-row (breeding), and two-row (genebank) subpopulations using the ggplot2 package (Wickham 2016; Wang & Zhang 2021).

## 4.2 Experimental design and phenotyping (disease-specific)

### 4.2.1 Scald experiment (Paper I)

*Growth conditions and experimental setup*

Genebank materials were evaluated for scald resistance under controlled greenhouse conditions at the Biotron facility, SLU (Figure 5). The experiment was conducted using an augmented block design, in which four commercial check cultivars were replicated across blocks, whilst genebank accessions were not replicated within each experimental run. Two independent experiments were performed. Plants were grown in individual pots, and disease evaluation was carried out at the seedling stage.


*Figure 5*. Crop growth conditions for evaluating scald resistance in spring barley under controlled environmental conditions at Biotron, Alnarp.

*Inoculum preparation and inoculation*

*R. graminicola* was collected from spring barley fields in southern Sweden. The isolate pathotype was not characterised. Inoculum was multiplied in vitro following an established protocol developed at the Department of Plant Breeding, SLU (Dhakal 2022). A conidial suspension was prepared at a concentration of $1.35 \times 10^6$ conidia $ml^{-1}$. Details about the inoculum

preparation procedures are described in Paper I. Following inoculation, plants were incubated under high humidity and dark conditions for 72h at a temperature of 16-17 °C. Thereafter, they were maintained under controlled greenhouse conditions, and disease development was monitored.

*Disease scoring and assessment*

Disease severity was assessed three times using a 0-10 scale (Figure 6), where 0 indicated no visible symptoms and 10 indicated the highest infection (Patil et al. 2003). Detailed descriptions of the data analysis and equations used in the calculation are presented in Paper I.


*Figure 6.* Scores used for *R. graminicola* infection with the increasing order of disease severity, 0 was asymptomatic and 10 was the highest severity of infection.

### 4.2.2 Net form net blotch experiment (Paper II)

*Growth conditions and experimental setup*

The experiment was performed under controlled greenhouse conditions at Lantmännen, Svalöv, Sweden. An alpha lattice design with three replicates was used, and the whole experiment was conducted twice. Disease severity was assessed at the seedling stage.

*Inoculum preparation and inoculation*

A cryopreserved *Ptt* spore suspension was provided by Chawade Lab, Department of Plant Breeding, SLU. The isolate originated from spring barley fields in southern Sweden, and isolate characterisation was not performed. The V8 agar was used to culture the pathogen, which was later incubated under alternating UV and white light (12/12 h) at 19 °C for 2 weeks to induce sporulation. The spore suspension was subsequently prepared by adjusting the concentration to 30,000 spores $ml^{-1}$, following an optimised

CIMMYT protocol (Gilchrist-Saavedra et al. 2006). The seedlings were inoculated by hand-spraying and then incubated at 20 °C under high humidity for 3 days (Figure 7). Following this, humidity was reduced to approximately 70% and maintained until symptom development, after which disease assessments were conducted. Detailed procedures are provided in Paper III.

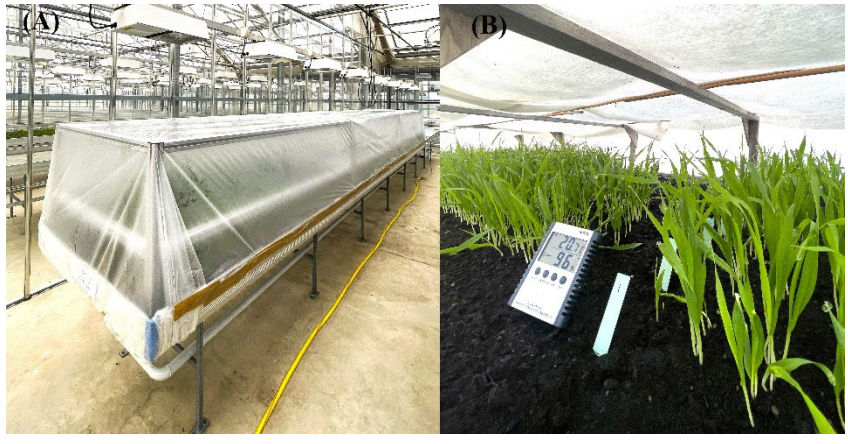


*Figure 7.* Inoculation conditions for net form net blotch disease evaluation. (A) Fully enclosed incubation chamber equipped with a humidifier to maintain high humidity. (B) Barley seedlings growing inside the chamber under monitored temperature and relative humidity.

### *Disease scoring and assessment*

Disease severity was assessed five times using a 1-10 scoring scale (Figure 8), where 1 indicated minimal infection and 10 indicated severe infection (Tekauz 1985; Jalli 2011). Detailed data analysis procedures are described in Paper II.

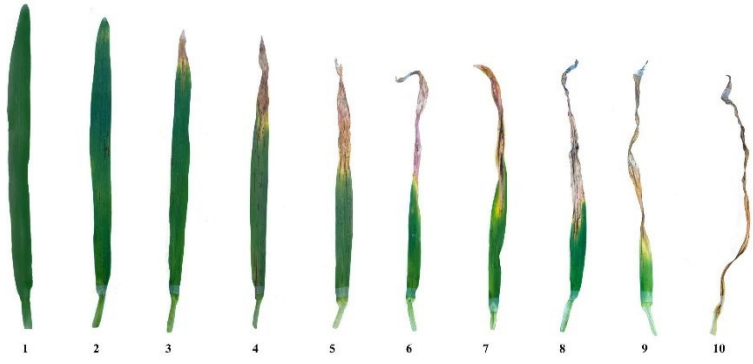


*Figure 8.* Scores used to evaluate *P. teres f. teres* infection where score 1 represented asymptomatic and 10 indicated the highest severity of infection.

*Field evaluation (cross-stage validation)*

In addition to the greenhouse experiment, NFNB resistance was evaluated under field conditions at two locations in Latvia and Lithuania. Trials were conducted with two replicates in Latvia and four replicates in Lithuania. Disease severity was assessed once at the adult-plant stage under natural infection conditions.

### 4.2.3 Powdery mildew experiment (Paper III)

*Growth conditions and experimental setup*

Powdery mildew resistance was evaluated under controlled-environmental conditions at Lantmännen, Svalöv, Sweden. The experiment was conducted using an alpha lattice design with three replicates and was performed twice. Seedlings were grown directly on greenhouse benches, and disease assessments were carried out at the seedling stage.

*Inoculum preparation and inoculation*

Powdery mildew inoculum was obtained from naturally infected spring barley leaves collected from barley-growing fields. Inoculation relied on field-collected material, and isolate characterisation was not performed. Given that *B. hordei (Bh)* is an obligate pathogen, it was cultured on living barley plants. The plants were inoculated twice and subsequently maintained under controlled growth conditions (Figure 9). Detailed inoculation procedures are presented in Paper II.


*Figure 9.* Crop growth conditions for evaluating powdery mildew resistance in spring barley advanced breeding lines. (A) Experimental set up. (B) Growing *Bh* on living spring barley plants to maintain viable inoculum.

### *Disease scoring and assessment*

Disease severity was examined three to four times per experiment, depending on disease progression, using a 0-4 scale (Figure 10), where 0 indicated no visible symptoms and 4 indicated maximum infection (Jørgensen & Wolfe 1994). Subsequent data analysis including the equations used in this study are provided in Paper III.


*Figure 10.* Disease scales for the powdery mildew disease evaluation, ranging from 0 to 4. Score 0 represents minimum infection (almost no infection), and 4 represents maximum infection (100%).

## 4.3 Phenotypic data processing

Disease scores collected over time were later summarised, and the area under the disease progression curve (AUDPC) was subsequently calculated using the trapezoidal method (Campbell & Madden 1990). Using these values as phenotypic data, the best linear unbiased estimates (BLUEs) and best linear unbiased predictions (BLUPs) were calculated using mixed linear models implemented in the META-R software (Alvarado et al. 2023). For the scald experiment, genotypic effects were considered as fixed, and BLUEs were estimated. For the powdery mildew and NFNB experiments, BLUPs were predicted by treating genotypic effects as random.

## 4.4 Genotypic data processing and analyses

### 4.4.1 Marker Information and quality control

All spring barley panels were genotyped using the 15K Illumina Infinium SNP array from TraitGenetics GmbH (SGS, Germany). Marker quality control was performed using TASSEL by removing SNPs with more than 10% missing values and minor allele frequency (MAF) below 0.05 (Bradbury et al. 2007). These filtering criteria were applied across all datasets used in Papers I-III. Given that GWAS analyses were conducted across different populations, the final number of retained markers varied among studies. Physical positions of markers were obtained from the Morex V3 reference genome, and marker annotations were retrieved from the publicly available Triticeae Toolbox and BARLEYMAP databases (Cantalapiedra et al. 2015; Blake et al. 2016; Mascher et al. 2021).

### 4.4.2 Genotypic datasets across studies

The genotypic data analysed in this thesis were obtained from various barley populations, including genebank germplasm and advanced breeding lines. Some populations were analysed independently, whereas others shared common genotypes which facilitated comparisons across diseases and analytical methods. These variations in dataset composition are critical for evaluating how GWAS and GP results compare across studies.

### 4.4.3 Linkage disequilibrium (LD) analysis

Linkage disequilibrium (LD) was evaluated using pairwise marker correlations ($r^2$) calculated in TASSEL with a sliding window of 50 SNPs. Analyses were conducted separately for individual subpopulations and combined populations depending on the study (Hill & Weir 1988; Bradbury et al. 2007). LD decay was then examined by relating $r^2$ values to the physical distance along each chromosome. Chromosome-specific LD decay distances were used to group nearby marker-trait associations (MTAs) into a single QTL and to evaluate the novelty of detected QTL relative to previously reported QTL (Visioni et al. 2020).

### 4.4.4 Genome-wide association studies (GWAS) analysis

GWAS analyses were performed using multi-locus models implemented in the GAPIT 3 package (Wang & Zhang 2021). For the scald experiment, both BLINK and FarmCPU models were applied, whereas BLINK was used for the powdery mildew and the NFNB experiments to identify MTAs (Liu et al. 2016; Huang et al. 2019). Depending on the study objectives, GWAS analyses were conducted on individual subpopulations and combined populations to detect population-specific and shared associations. Significance thresholds were determined separately for each dataset using the Bonferroni correction and the false discovery rate (FDR) procedures, based on the number of retained markers. Details regarding GWAS workflows and thresholds are described in the respective papers.

## 4.5 Downstream analyses (disease-specific)

### 4.5.1 Scald: favourable allele analysis

Estimated marginal means of alleles at significant MTAs were calculated using the Emmeans package (Searle et al. 1980). Favourable alleles were identified based on their effects, and their combined contribution was evaluated to identify genotypes with superior resistance profiles. Full statistical details are provided in Paper I.

### 4.5.2 Net form net blotch: genomic prediction strategies

GP was used to evaluate NFNB resistance across individual and combined populations. Prediction accuracy was assessed using multiple cross-validation approaches that reflected different breeding scenarios, including within-population, between-population, and subset-population validations with varying training population compositions.

Predictive ability was calculated by correlating genomic estimated breeding values (GEBVs) and observed phenotypic values. These analyses were used to assess the transferability of prediction models across populations and to examine the effects of population composition on GP performance. Detailed descriptions of the models and validation schemes are provided in Paper II.

### 4.5.3 Powdery mildew: genomic prediction strategies

For powdery mildew, multiple selection strategies were evaluated using the advanced breeding panel, with analyses carried out separately for two-row and six-row subpopulations. Weighted marker-assisted selection (wMAS) was performed based on GWAS-identified loci by combining marker effects to calculate genetic merit scores for individual genotypes.

GP was performed in parallel with genome-wide markers using two strategies: (i) models fixing GWAS-significant markers together with genome-wide random effects, and (ii) standard GP models treating all markers as random effects. Predictive ability accuracy was assessed using repeated cross-validation. Genotype selection based on phenotypic evaluation, wMAS-derived genetic merits, and GEBVs was compared to assess similarities and differences among selection strategies. Methodological details are provided in Paper III.

### 4.5.4 Cross-stage validation using field data for net form net blotch resistance

Field phenotypic data collected from two locations with replicated trials were summarised as BLUPs using mixed linear models, consistent with the analytical workflow applied to greenhouse data. The key difference between the greenhouse and field evaluations was the crop growth stage that resistance was evaluated at: seedling under controlled conditions versus adult-plant stage under field conditions.

GP models developed using seedling-stage resistance were used to generate GEBVs, which were correlated with adult-plant field BLUPs using

Spearman's rank correlation. This analysis aimed to assess cross-stage transferability rather than to train independent GP models based on field data.

## 4.6 Integration of study populations and analytical approaches

The three studies conducted in this thesis used GWAS analyses across all diseases to detect loci associated with resistance to scald, powdery mildew, and NFNB. This approach aims to investigate the genetic basis of these resistance traits. Nevertheless, the application of GP depends on the specific objectives of each study, leading to variations across populations.

For powdery mildew, four selection strategies were evaluated within advanced breeding populations. This included a selection strategy based on phenotypic performance, wMAS, GP models that used GWAS-significant markers as fixed effects, and standard GP models that treated all markers as random effects. Regarding NFNB, several GP models were used. Their predictive performance was later evaluated across different cross-validation scenarios, including both within-population, subset-population, and between-population predictions.

# 5. Results and discussion

## 5.1 Overview of genetic architecture of disease resistance across populations

Principal component analysis (PCA) showed clear genetic structure among the study populations (Figure 11). The six-row (breeding) subpopulation formed a distinct cluster, whilst the two-row (breeding) and two-row (genebank) subpopulations were more widely distributed, indicating greater genetic diversity. These patterns suggest differences in genetic diversity and background among populations.

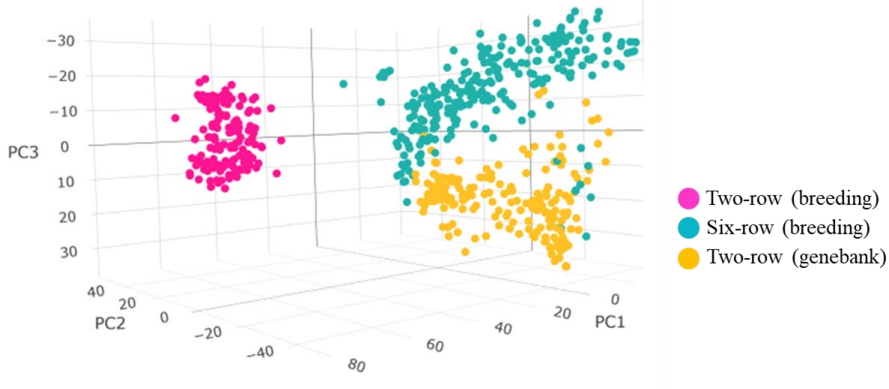


*Figure 11.* Principal component analysis (PCA) showed clear genetic differences among the study breeding panels.

Resistance to the three foliar diseases studied in this thesis was governed by major-effect loci as well as polygenic background, with clear differences observed among diseases and genotype panels. These differences reflect variations in breeding history, allelic diversity, and historical selection pressures, but do not necessarily indicate biological differences among the host-pathogen systems. Similar population-dependent resistance patterns have been reported in previous cereal disease-resistance studies (Sneller et al. 2009; Alemu et al. 2021; Zakieh et al. 2021).

In general, resistance in diverse genebank panels was distributed across the genome through cumulative allele effects, whereas introgressed major resistance loci played a more important role in advanced breeding panels. The detection of resistance loci further depended on population size, allele

frequency, and genetic background, emphasising the importance of population composition when interpreting GWAS-derived resistance signals. Table 1 summarises QTL detected for the evaluated diseases which were identified across barley populations. In the table, QTL are classified as major or minor based on the proportion of phenotypic variance explained (PVE). When the PVE of a QTL was greater than 10%, it was classified as major-effect loci, whereas if the PVE was less than 10%, the QTL was classified as minor-effect loci.

Table 1. *Summary of QTL detected through GWAS associated with scald, PM, and NFNB across different barley populations*

| Disease | Population | QTL | Chromosome | Effect class |
|---|---|---|---|---|
| Scald | Genebank | QTL_3H_1 | 3H | Major |
| Scald | Genebank | QTL_3H_2 | 3H | Minor |
| Scald | Genebank | QTL_3H_3 | 3H | Minor |
| Scald | Genebank | QTL_6H_1 | 6H | Minor |
| Scald | Genebank | QTL_7H_1 | 7H | Major |
| Scald | Genebank | QTL_7H_2 | 7H | Minor |
| Scald | Genebank | QTL_7H_3 | 7H | Minor |
| PM | Six-row | QTL_1H_1 | 1H | Minor |
| PM | Six-row | QTL_4H_3 | 4H | Major |
| PM | Six-row | QTL_5H_3 | 5H | Minor |
| PM | Two-row | QTL_4H_3 | 4H | Major |
| NFNB | Combined1,3 | QTL_1H_1 | 1H | Minor |
| NFNB | Genebank | QTL_1H_2 | 1H | Major |
| NFNB | Combined2 | QTL_1H_2 | 1H | Major |
| NFNB | Six-row | QTL_1H_3 | 1H | Major |
| NFNB | Combined3 | QTL_1H_4 | 1H | Minor |
| NFNB | Two-row | QTL_3H_1 | 3H | Minor |
| NFNB | Genebank | QTL_3H_2 | 3H | Minor |
| NFNB | Combined3 | QTL_4H_1 | 4H | Minor |
| NFNB | Six-row | QTL_6H_1 | 6H | Major |
| NFNB | Two-row | QTL_6H_2 | 6H | Major |
| NFNB | Combined1,3 | QTL_6H_2 | 6H | Minor |
| NFNB | Two-row | QTL_6H_3 | 6H | Minor |
| NFNB | Six-row | QTL_6H_4 | 6H | Minor |
| NFNB | Combined1,3 | QTL_6H_5 | 6H | Minor |
| NFNB | Combined1 | QTL_6H_6 | 6H | Major |
| NFNB | Combined3 | QTL_7H_1 | 7H | Minor |

The combined population includes GWAS analyses conducted on different combinations of subpopulations, including two-row + six-row breeding lines, two-row breeding lines + genebank accessions, and all genotypes combined. Scald: scald disease; PM: powdery mildew disease; NFNB: net form net blotch disease. As QTL detected in these analyses differed among combination types, these results are summarised under a single combined category to reflect the overall effect of increased population size and allelic diversity on QTL detectability.

### 5.1.1 Scald resistance architecture in diverse barley germplasm

Seven loci were significantly associated with scald resistance, three of which were novel and extended the known genetic basis of scald resistance beyond well-characterised regions. These loci explained a high proportion of phenotypic variance, although the estimates varied depending on the statistical model that was applied.

Of the seven detected loci, four overlapped with previously reported scald resistance regions, including two that colocalised with the well-known *Rrs1* complex (Lehnackers & Knogge 1990), supporting chromosome 3H as a recurrent region for scald resistance. Moreover, QTL detected on chromosome 7H overlapped with loci previously identified for resistance to other major barley diseases, including barley leaf stripe (Tacconi et al. 2001) and barley stem rust (Brueggeman et al. 2002). This result demonstrates that resistance-associated signals in this region recur across multiple disease studies.

### 5.1.2 Divergence of powdery mildew resistance in two-row and six-row advanced breeding lines

Powdery mildew resistance exhibited contrasting genetic architectures between the two-row and six-row subpopulations. In the two-row panel, resistance was largely driven by major-effect loci, particularly those associated with the well-characterised *mlo* and *mla* loci. This pattern reflects the long-term breeding focus on powdery mildew resistance in two-row barley due to the importance it holds within the malting industry (Miralles et al. 2021). Continuous introgression and selection of these loci led to the repeated detection of strong resistance signals and a limited contribution of polygenic background. In contrast, resistance in the six-row panel involved both major and minor-effect loci distributed across the genome, reflecting broader allelic diversity and less intensive fixation of specific resistance genes.

A total of 21 QTL were identified across the two subpopulations, many of which colocalised with previously reported resistance genes and major QTL, whilst eight represented potentially novel genomic regions. Some detected QTL overlapped with loci previously associated with adult-plant resistance, suggesting their potential to broaden resistance across developmental stages. Validation of the novel loci in independent populations or across multiple

locations would be beneficial to confirm their stability and breeding relevance.

### 5.1.3 Population-dependent detection of net form net blotch resistance loci

GWAS analyses for NFNB resistance identified 14 QTL across individual subpopulations and combined populations, five of which colocalized with previously reported resistance loci whilst the remaining nine were novel. The detection of resistance loci differed among populations, indicating population-dependent genetic architectures associated with NFNB resistance. QTL detected on chromosomes 3H, 4H, and 6H were reported to confer seedling-stage and adult-plant resistance in earlier studies (Clare et al. 2021; Rehman et al. 2025). Notably, a genomic region (QTL_6H_5) colocalising with the well-known *Rpt5* locus and exhibiting both resistance and susceptibility against NFNB disease (Clare et al. 2021; Richards et al. 2024) was detected only in the combined populations (combined 1 and combined 3),. This suggests that increased population size and allelic diversity improved the detectability of this locus in the GWAS analysis.

## 5.2 Genomic prediction and selection strategies for disease resistance

### 5.2.1 GWAS-informed genotype selection for scald resistance

Following QTL detection, genotypes were grouped based on their favourable allele combinations, and their BLUE (AUDPC) values were compared. In general, genotypes carrying more favourable alleles exhibited lower BLUE values, indicating reduced disease severity (Figure 12). The lowest disease incidences were observed in genotypes combining favourable alleles across multiple loci.

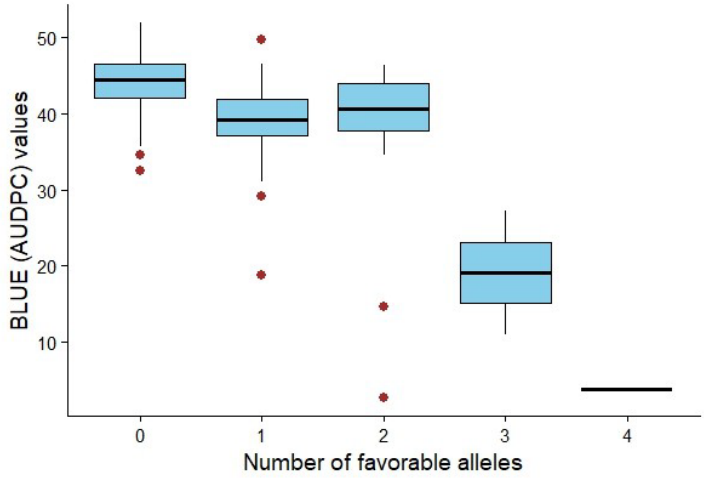


*Figure 12.* BLUE (AUDPC) values of genotypes grouped by their favourable allele combinations for scald resistance.

Despite this overall trend, substantial variation was observed within each allele group, indicating that favourable allele combinations alone did not fully explain phenotypic performance. Genotypes carrying fewer favourable alleles often possessed similar resistance levels to those carrying more, suggesting that additional genetic effects could be presented beyond the favourable alleles at detected QTL or unfavourable alleles at other loci modulated resistance expression. Overall, these results support an additive genetic architecture for scald resistance, with resistance progressively increasing as favourable alleles accumulate, but with considerable influence from the broader genetic background.

### 5.2.2 Comparative performance of genomic selection strategies for powdery mildew resistance

Genotypes selected using phenotypic evaluation and genomic approaches were compared to assess concordance among selection strategies for powdery mildew resistance. Strong correlations between genetic merit estimates and BLUP (AUDPC) values were observed in both the two-row (0.76) and six-row (0.73) subpopulations, indicating that GWAS-significant markers captured a substantial proportion of phenotypic variation underlying resistance.

Genomic prediction analyses revealed clear differences in model performance between the Standard GP and GWAS-incorporated GP models

(Figure 13). In both subpopulations, GP models incorporating GWAS-significant markers as fixed effects achieved higher and more stable predictive abilities across iterations, whereas Standard GP model which included all genome-wide markers as random effects showed lower predictive ability and greater variability. This improvement likely reflects the ability of GWAS-incorporated models to capture the effects of major loci without shrinkage, subsequently enhancing prediction accuracy when loci with large effects are present.

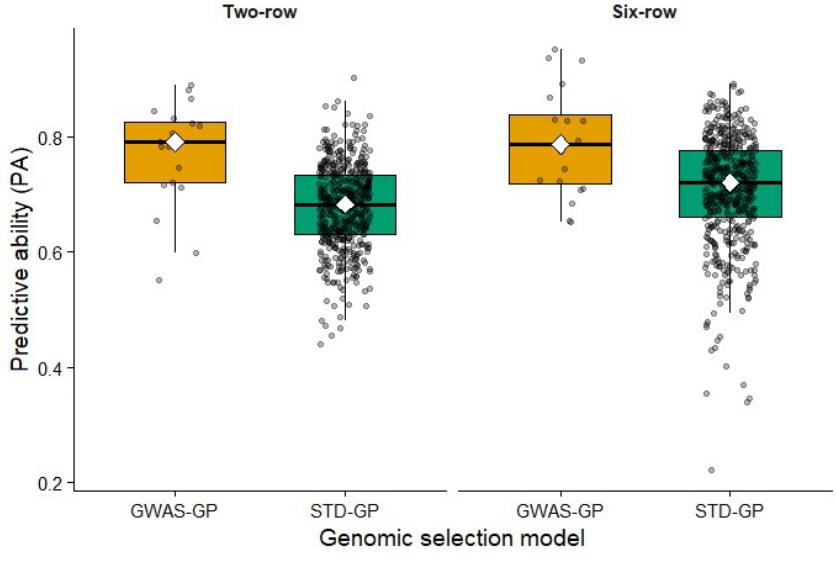


*Figure 13.* Predictive ability (PA) for standard genomic prediction (STD-GP) and GWAS-incorporated genomic prediction (GWAS-GP) models in two-row and six-row barley subpopulations. Each point represents a single model iteration, whilst boxplots summarise the median and interquartile range of PA values. PA values of the GWAS-GP model were obtained from 20 random iterations, whilst PA values for the STD-GP model were obtained from 500 iterations.

Genotype selection overlaps among phenotypic, wMAS, and GP strategies were limited under stringent selection thresholds, with only a small subset of genotypes consistently selected across methods (Figure 14). In the two-row subpopulation, wMAS predominantly selected genotypes carrying the favourable allele at a single detected locus which explained the large number of genotypes uniquely selected by this approach. In contrast, the six-row subpopulation displayed a more complex genetic architecture, resulting in a smaller wMAS-exclusive group. When the selection threshold was relaxed

from 10% to 20%, greater overlap was observed among the selection strategies, reflecting the inclusion of genotypes with moderate resistance and mixed genetic backgrounds.

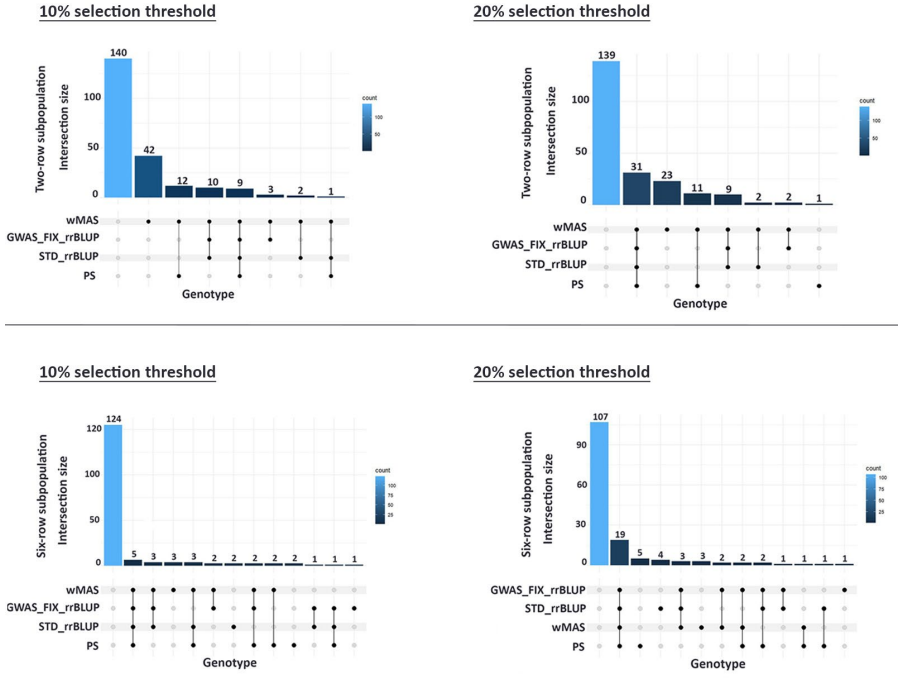


*Figure 14.* UpSet plots illustrating the overlap of genotypes selected across different selection strategies in the two-row and six-row barley subpopulations under 10% (left) and 20% (right) selection thresholds. Selection strategies include phenotypic selection (PS), weighted marker-assisted selection (wMAS), standard genomic prediction (STD-rrBLUP), and GWAS-informed genomic prediction (GWAS-FIX-rrBLUP). Bars indicate the number of genotypes selected by individual methods or shared across multiple methods. Black circles represent methods that selected a genotype, whilst grey circles represent methods that did not. Connecting lines indicate genotypes jointly selected by more than one strategy.

Overall, these results indicate that the effectiveness of selection strategies depends on the underlying genetic architecture. Whilst wMAS is efficient for short-term selection in populations dominated by major-effect loci, GP is more effective, particularly when incorporating GWAS-driven information to capture both major and polygenic components of resistance in complex populations.

### 5.2.3 Population-dependent genomic prediction of net form net blotch resistance

GP analyses demonstrated that prediction accuracy for NFNB resistance was strongly dependent on population composition and cross-validation strategy (Figure 15). Within-population predictive abilities ranged from 0.37 to 0.71, with the highest value (0.71) observed for the six-row population and combined (two-row + six-row) populations. The six-row population was characterised by strong LD, moderate genetic diversity, and high heritability whereas the combined population achieved a high PA due to its larger training size which subsequently improved model stability. Similar results have been reported in earlier work (Daetwyler et al. 2010; Berro et al. 2019). In contrast, prediction accuracy in the genebank population was substantially lower (0.37), consistent with its lower heritability, weaker LD, and smaller sample size. The relative contribution of these factors was schematically illustrated based on observed population characteristics and predictive performance, rather than formal variance decomposition.

The accuracy of between-population prediction (0.28-0.60) was lower than within-population predictions, reflecting the limited transferability of marker effects across genetically distinct populations. The highest accuracy (0.60) was obtained when the two-row population was used to predict the six-row population, whereas prediction in the opposite direction resulted in reduced accuracy (0.34). This asymmetry suggests that whilst the six-row population enables accurate within-population prediction, possibly due to its homogeneous genetic architecture, its marker effects generalise less effectively to populations with differing LD structure and genetic relatedness.

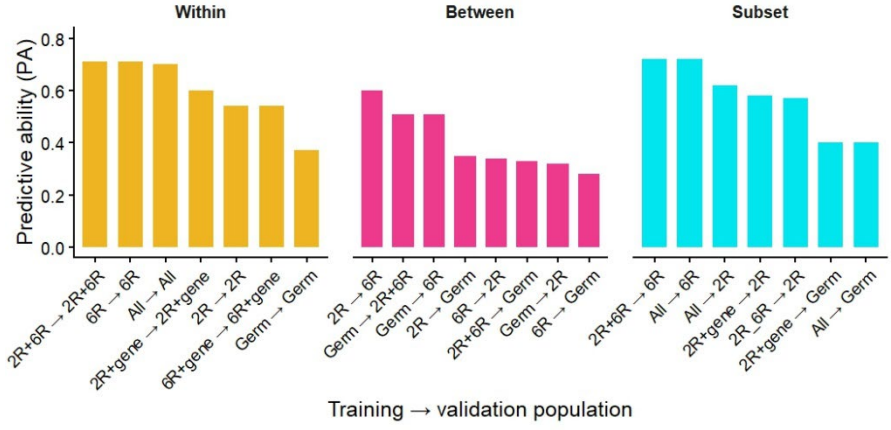


*Figure 15.* Predictive ability (PA) of genomic prediction under different cross-validation strategies. Panels show within-population, between-population, and subset-population prediction scenarios. Bars represent PA values for each training → validation population combination. Within-population PA values were obtained from 500 iterations, subset-population PA values from 20 iterations, and between-population PA values from single analyses using entire subpopulations as training and validation sets.

Subset-population cross-validation produced prediction accuracies (0.40-0.72) comparable to within-population prediction, reflecting increased relatedness and shared LD structure between training and validation sets. Higher accuracies were observed when training populations exhibited shorter LD decay than the validation subsets, indicating more efficient transfer of marker effects from compact LD blocks to broader LD ones. Similar population-dependent patterns have been previously reported (Liu et al. 2015; He et al. 2023). Overall, these results indicate that GP performance for NFNB resistance is driven by a combination of heritability, LD structure, genetic relatedness, and population composition. Solely increasing the training population size was insufficient to overcome unfavourable genetic conditions. Instead, prediction accuracy was maximised when cross-validation strategies and training populations were aligned with the underlying genetic architecture of the target population.

### 5.2.4 Transferability of greenhouse-based genomic prediction to field resistance for net form net blotch

GP models trained on seedling-stage NFNB resistance of genebank accessions were validated against adult-plant resistance measured in field trials to assess the relevance of genomic signals detected under controlled greenhouse conditions for field resistance. The average predictive ability within the greenhouse dataset was approximately 0.35, ranging from 0.04 to 0.65 and thus indicating moderate genomic signal for seedling-stage resistance. Correlation between GEBVs and adult-plant field BLUPs was weaker but statistically significant (Spearman's $\rho \approx -0.25$, $p < 0.01$), accounting for about 6% of the shared variance. These results show that greenhouse-derived GPs have partial but limited transferability to adult-plant resistance.

The reduced association indicates that seedling-stage and adult-plant resistance are controlled by different genetic architectures. Previous studies investigating barley and other cereals have shown that QTL detected at early growth stages are not always expressed at later stages (Amezrou et al. 2018; Visioni et al. 2020). As adult-plant resistance is also largely influenced by environmental conditions, this consequently makes estimation challenging (Vo Van-Zivkovic et al. 2025). The present results are consistent with these findings, demonstrating that greenhouse phenotyping only partially captures the genetic variation relevant to field resistance.

GP accuracy depends on how well the phenotyping conditions of the training population represent the target breeding context. Factors such as trait heritability, genetic architecture, and relatedness between training and validation sets influence prediction accuracy (Crossa et al. 2017; Alemu et al. 2024). GP performed reasonably well when both training and validation used greenhouse phenotypes, but accuracy decreased when greenhouse-trained models were applied to field-based adult-plant resistance. The weak correlation between greenhouse-based predictions and field-derived BLUPs indicates limited transferability across phenotyping conditions. Thus, greenhouse phenotyping is useful for early-stage screening but cannot replace field-based evaluation when breeding objectives focus on adult-plant resistance. The results of this thesis support an integrated approach in which greenhouse screening and field evaluation are used together rather than interchangeably.

# 6. Conclusions

The genetic basis of major foliar diseases of spring barley, namely scald, net form net blotch, and powdery mildew, was examined using GWAS and GP approaches across diverse genotype panels. Paper I revealed both novel and known QTL linked to scald resistance, by utilising the Nordic barley genebank panel; this highlights the significance of genetic diversity in resistance breeding programmes. Paper II assessed how well GP worked for NFNB disease across different validation scenarios. The results showed that predictive ability was significantly affected by LD structure, heritability, and the genetic compatibility between training and validation populations. Paper III demonstrated that GP models that incorporated GWAS-identified markers improved prediction of powdery mildew resistance and underscored their potential for use in breeding programmes. Overall, this work identified the importance of population composition, crop developmental stage, and analytical strategy for QTL detection, genotype selection, and prediction performance. Based on that, the following conclusions were drawn:

- Resistance to all three diseases was controlled by multiple loci, with both the number and effect size of detected QTL varying among diseases and populations (Papers I-III).
- Population composition, including genetic diversity, distribution of allele frequency, and LD patterns, strongly influenced both the detection of resistance loci and the stability of their estimated effects (Paper II).
- Although combining populations with different genetic backgrounds increased training population size, prediction performance became less consistent due to increased genetic heterogeneity and reduced transferability of marker effects (Paper II).
- Subset-population validation showed that prediction accuracy comparable to within-population prediction could be achieved when training and validation sets were genetically compatible and shared related genotypes (Paper II).

- Adding GWAS-preselected markers into GP models improved prediction as it included both major-effect loci and the overall polygenic effects of genome-wide markers (Paper III).
- When stringent selection thresholds were used, the genotypes identified through genomic prediction and wMAS only partially overlapped. This overlap expanded as the selection criteria were relaxed, suggesting that the selection signals complemented rather than conflicted with each other (Paper III).
- Validation across various growth stages revealed restricted transferability of seedling-stage genomic signals to adult-plant resistance in field conditions, and QTL identified in greenhouse contexts were not detected in field observations.

# 7. Future perspectives

The resistance loci identified in this thesis were detected in specific populations and experimental conditions; thus, their consistency should be examined across various environments, populations, and growth stages for broader relevance. Comparing seedling and adult-plant resistance is particularly helpful in determining whether associated genomic signals are maintained across developmental stages.

GP accuracy is determined by population composition, LD structure, genetic relatedness between the training and validation sets, and the correspondence between training phenotypes and the intended selection environment. Optimising the design of the training population rather than simply increasing its size can provide more stable and transferable predictive outcomes.

Expanding the approach from a single disease focus to one that includes multi-trait GP, such as combining disease resistance with agronomic traits like yield, could support more balanced selection decisions in breeding programmes. Furthermore, combining seedling-stage screening with adult-plant field evaluation may improve the practical application of genomic prediction in breeding.

As most of the germplasm evaluated in this thesis was comprised of Swedish landraces, incorporating barley material with broader genetic backgrounds in future studies will improve inference and applicability. Future research that incorporates biological insights with population-specific genomic approaches is expected to further support disease-resistance breeding in spring barley.

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# Popular science summary

Barley is an important cereal crop used for various purposes, such as animal feed and malting. However, fungal diseases such as scald, powdery mildew, and net form net blotch (NFNB) reduce its yield and grain quality. Developing resistant varieties to control these diseases is one of the most sustainable ways to decrease reliance on chemical fungicides.

Disease resistance in barley is often controlled by many genes with small effects, a form known as quantitative resistance. Although this type of resistance is usually more durable, it is also more complex to study and apply in breeding. In this thesis, resistance to three major barley diseases was investigated using diverse spring barley population, including genebank landraces and advanced breeding lines. Both disease symptoms and DNA markers were analysed to understand how genetic differences influence resistance.

The results showed that resistance is controlled by multiple genomic regions that vary between diseases and barley populations. This means that genetic background plays an important role in determining which resistance genes are effective. The study also examined whether resistance can be predicted using DNA information alone. Prediction accuracy strongly depended on how closely the plants that were used during the training of the model resembled the plants in the target population. Greenhouse experiments provided valuable early-stage screening and helped to identify important resistance signals. However, resistance observed at the seedling stage under greenhouse conditions did not always reflect resistance expressed later in the field, highlighting the need to combine greenhouse and field evaluations. Overall, this thesis enhances understanding of disease resistance in barley and supports the development of healthier and more sustainable barley crops.

# Populärvetenskaplig sammanfattning

Korn är en viktig spannmålsgröda som används till djurfoder och maltning. Svampsjukdomar som skållning, mjöldagg och nätfläckar minskar dock avkastning och spannmålskvalitet. Att utveckla resistenta sorter för att bekämpa dessa sjukdomar är ett av de mest hållbara sätten att minska beroendet av kemiska svampmedel.

Resistens i korn styrs ofta av många gener med små effekter, en form som kallas kvantitativ resistens. Även om denna typ av resistens vanligtvis är mer varaktig, är den också mer komplex att studera och tillämpa i avel. I denna avhandling undersöktes resistens mot tre stora kornsjukdomar med hjälp av olika vårkornspopulationer, inklusive genbanksländraser och avancerade avelslinjer. Både sjukdomssymptom och DNA-markörer analyserades för att förstå hur genetiska skillnader påverkar resistens.

Resultaten visade att resistens kontrolleras av flera genomiska regioner som varierar mellan sjukdomar och kornpopulationer. Detta innebär att genetisk bakgrund spelar en viktig roll för att bestämma vilka resistensgener som är effektiva. Studien undersökte också om resistens kan förutsägas enbart med hjälp av DNA-information. Prediktionens noggrannhet berodde starkt på hur nära de växter som användes under träningen av modellen liknade växterna i målpopulationen. Växthusexperiment gav värdefull screening i tidigt skede och hjälpte till att identifiera viktiga resistenssignaler. Resistens som observerades i plantstadiet under växthusförhållanden återspeglade dock inte alltid resistens som uttrycktes senare i fält, vilket belyser behovet av att kombinera växthus- och fältutvärderingar. Sammantaget förbättrar denna avhandling förståelsen av sjukdomsresistens hos korn och stöder utvecklingen av hälsosammare och mer hållbara korngrödor.

Resilient korn: Att förutsäga hållbar sjukdomsresistens under miljömässig och patogenmångfald

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# I

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RESEARCH

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# Harnessing novel genetic markers for scald resistance from gene bank spring barley genotypes

Su Myat Noe[1], Johanna Åstrand[2], Mustafa Zakieh[1], Pawan Kumar Singh[3], Eva Johansson[1] and Aakash Chawade[1*]

## Abstract

**Background** Scald caused by *Rhynchosporium graminicola* is a common foliar disease affecting barley production worldwide. Identifying and utilizing scald resistance genes and quantitative trait loci (QTL) to develop barley cultivars with durable and effective resistance to scald is crucial.

**Results** In the present study, we evaluated 275 spring barley genotypes together with 4 commercial check cultivars under controlled conditions and examined the underlying genetics of scald resistance in these genotypes. A significant genetic variation ($P$ value < 0.0001) for scald resistance was observed among the tested barley germplasms. A genome-wide association study (GWAS) identified eight markers–trait associations (MTAs) forming seven QTL located on chromosomes 3H, 6H, and 7H, of which three are novel. The allelic effects of these MTAs were further examined, and favorable alleles associated with scald resistance were identified.

**Conclusions** The identification of QTL for scald resistance, along with favorable allele identification, will be crucial for marker-assisted breeding programs. These findings will facilitate the development of new scald-resistant cultivars and contribute to the sustainability of barley production. Further studies, such as fine-mapping of candidate genes within these identified QTL regions, will help to narrow down the potential causative genetic variants and understand their functional effects on scald resistance.

**Keywords** GWAS, Barley, Scald, AUDPC, Biotic stress

## Background

Barley (*Hordeum vulgare* L.) is an annual grass in the Poaceae family. Despite its adaptability to various environments, barley is cultivated primarily in temperate regions [1]. Globally, barley is the fifth most produced cereal crop in terms of production acreage [2]. In 2020/2021, 160 million metric tons of barley were produced, with the European Union being the most productive region with 53 million metric tons [3].

Improving crop yield is a primary objective of plant breeding programs; however, reaching the full yield potential is severely prohibited by abiotic (such as drought and temperature) and biotic (pests and diseases) constraints [4–6]. Plant diseases such as net blotch, scald, leaf blotch, brown rust, and powdery mildew significantly affect grain yield, quality and the biomass harvested for feed [7]. Among these diseases, scald can cause 30% yield loss and reduce grain quality [8]. The causal pathogen of scald disease is *Rhynchosporium graminicola* (formerly

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known as *R. commune*), a hemibiotroph fungus. It overwinters on plant residues, seeds, and soil, where the first two serve as the primary sources of inoculum and sporulation [9].

Eleven major resistance genes and several quantitative trait loci (QTL) associated with scald resistance have been identified and mapped as summarized in Supplementary Table S1. Among these, *Rrs1* was the first scald resistance gene discovered in barley and *Rrs18* is the most recent scald resistance gene, the latter has been mapped on Chr 6H. Additional examples are *Rrs4* mapped on Chr 3H in cultivated barley and *Rrs13* which was reported in an interspecific cross between *H. vulgare* and *H. spontaneum* [10–21]. Although multiple resistance genes for scald disease have been discovered and subsequently used in breeding programs, the pathogen evolve and develop virulence to overcome resistance genes. The defense system in plants are overcome due to several biotic and abiotic selection pressures, such as mutations (gene-for-gene interactions between pathogens and host plants), climate change, and fungicide application [22]. One way to delay the breakdown of disease resistance is by broadening the genetic basis of host resistance through the pyramiding of resistance genes together with QTL with minor- to moderate effect, which may also result in more durable and effective resistance to scald disease [23]. Therefore, the discovery of qualitative and quantitative resistance genes for scald disease resistance from various genetic resources, such as diverse germplasms from gene bank, is crucial.

As of 2019, 1,750 gene banks worldwide have collected 7.5 million germplasms for several plant species, including landraces, wild relatives, mutants, and genetic resources, which are conserved in different forms, such as seeds and other plant parts, including shoots and pollens [24]. Landraces and wild relatives likely exhibit tolerance and resistance to biotic stresses, providing potential benefits for modern cultivars. Recent advances in genotyping technologies and reduced costs associated with sequencing allow researchers to effectively explore diversity in gene bank accessions. Genome-wide association studies (GWAS) have emerged as a powerful tool in identifying the genetic basis of complex traits, including disease resistance in barley. For example, a GWAS conducted using a worldwide barley collection, including 277 landraces, identified 15 QTL for net form net blotch (NFNB) (*Pyrenophora teres* f. *teres*) resistance, four of which were newly reported QTL [25]. Another association mapping study used wild and landrace barley populations collected across Türkiye and detected four and ten QTL associated with SFNB spot form net blotch (SFNB) and NFNB, respectively [26]. A GWAS study identified two new major QTL for powdery mildew resistance from a mixture of 696 barley accessions where more than half of the mixture consisted of wild type and landraces [27]. In addition, an association study using Ethiopian landraces identified 17 marker-trait associations (MTAs) across seven chromosomes associated with barley scald disease [28]. Moreover, a GWAS analysis was conducted with 131 Scottish Bere barley landraces resulting in the detection of a number of genomic regions associated with scald resistance and among them, four QTL were novel of which candidate gene were identified [29]. A recent genetic association study identified 22 QTL, some of which some were novel and associated with scald resistance. The synchronized use of advanced technologies like GWAS and the rich genetic diversity enables precise identification of resistance genes and QTL, enabling targeted breeding strategies to enhance crop resilience against biotic stresses [9].

The current study utilized a diverse panel of 275 barley genotypes from the NordGen gene bank to evaluate scald resistance by artificial inoculation under greenhouse conditions. The objectives of the study were to i) evaluate the scald resistance of the tested gene bank accessions, ii) examine the genetic makeup and patterns of genetic association within the gene bank accessions, iii) identify QTL associated with scald resistance from the current barley germplasm, and iv) compare these with previous studies to identify novel findings and resources for breeding programs.

## Results

### Phenotypic variation among the tested barley accessions

Pearson correlation coefficients were computed to assess the consistency between the two experiments. A moderate correlation ($r = 0.61$) was observed between the two experiments. Meanwhile, the correlations between the BLUE values (estimated from AUDPC data across both experiments) and individual AUDPC observations were strong, with $r = 0.87$ for BLUE vs. AUDPC in experiment 1 and $r = 0.90$ for BLUE vs. AUDPC in experiment 2. These strong correlations confirmed that the BLUE values reliably capture genotype performance based on AUDPC.

The values for BLUE ranged from 2.59 to 51.96, indicating a left-skewed frequency distribution where lower values suggest greater genotype resistance. One hundred and sixty-five genotypes had a BLUE greater than the BLUE mean (42.6), while the remaining 113 genotypes had a lower BLUE than the mean (Fig. 1). The susceptible check cultivars Freja and Ingrid attained BLUE values of 44.94 and 44.15 (above the mean), respectively. In contrast, the moderately resistant check cultivars Laureate and RGT Planet achieved BLUE values of 37.77 and 39.3 (below the mean), respectively. Analysis of variance revealed a high significant

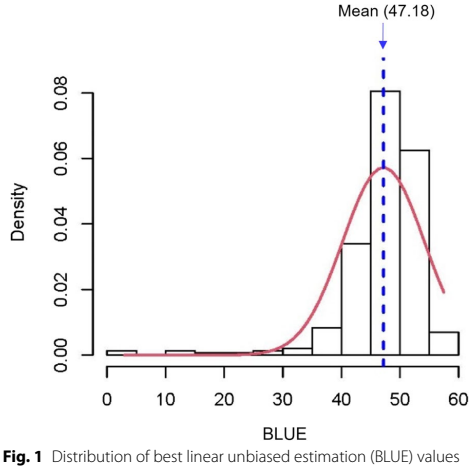


**Fig. 1** Distribution of best linear unbiased estimation (BLUE) values for scald disease in the spring barley population. The mean BLUE of the total population was 47.18. The population was skewed towards higher infection scores indicating high susceptibility in the germplasm

phenotypic variation among the studied genotypes (*P* value < 0.0001) and a broad-sense heritability of 0.73 was observed. The calculated BLUE values are shown in Supplementary Table S2. Examples of the phenotypic differences among the tested barley genotypes, along with the resistant and susceptible check cultivars, are shown in Fig. 2A-D. Figure 2A illustrates two genotypes with low BLUE, LOFA and ST-13947, while Fig. 2B shows two genotypes with high BLUE, Mitja and KVL211. Figure 2C illustrates the moderately resistant checks—Laureate and RGT planet—and Fig. 2D demonstrates the susceptible checks Ingrid and Freja.

#### Linkage disequilibrium

Linkage disequilibrium (LD) decay was estimated from 10,151 SNP markers and resulted in a total number of marker pairs on each chromosome varying from 48,522 (Chr 3H) to 96,216 (Chr 5H), with a total of 496,319 marker pairs in the whole genome. The average correlation coefficient ($r^2$) across the seven barley chromosomes ranged from 0.15 to 0.23, while the overall genome-wide $r^2$ value was 0.17. Overall, 61% of the total marker pairs across the whole genome were in significant LD, with an average $r^2$ of 0.17 at the 0.001 significance level (Table 1). The LD half decay in kilobase pairs (bp) for each chromosome varied from 1,449,455 bp (Chr 1H) to 4,427,179 bp (Chr 6H) (Fig. 3; Supplementary Fig. 2), and at the whole-genome level, the maximum LD decay was observed at a $r^2$ value of 0.46 and reached its half decay (2,083,171 bp) when $r^2$ reached 0.0.21 (Supplementary Fig. 2).

#### GWAS to identify QTL associated with scald resistance

GWAS was conducted utilizing BLUE as phenotypic data and 10,151 SNP markers as genotypic data using two multi-locus models, BLINK and FarmCPU. These models together identified eight markers–trait associations (MTAs) corresponding to seven QTL (Table 2; Fig. 4A-D)—three located on Chr 3H, one on Chr 6H, and three on Chr 7H. The BLINK model identified five MTAs (Fig. 4A and C), which accounted for 54.4% of the total phenotypic variance. On the other hand, the FarmCPU model identified six MTAs (Fig. 4B and D), which explained 33.9% of the phenotypic variation in the tested population (Table 2). The first QTL identified on Chr 3H was QTL_3H_1 at 424.23 Mbp, and the second QTL (QTL_3H_2) was located 17.9 Mbp away from the first QTL. The third detected QTL (QTL_3H_3) was located at 450.44 Mbp (26.2 Mbp and 8.23 Mbp away from the first and second QTL, respectively). On Chr 6H, a single QTL (QTL_6H) located at 527.21 Mbp was detected. Three QTL were detected on Chr 7H: QTL_7H_1 (5.4 Mbp), QTL_7H_2 (11.1 Mbp) and QTL_7H_3 (621.55 Mbp) (Fig. 4). Significant markers were assigned to the QTL based on their chromosomal LD half-decay estimates (1.4—4.4 Mbp across seven chromosomes) [30].

After identifying QTL, we further examined the allelic effect on the observed BLUE by performing an additional post hoc test (Tukey's HSD test). The test revealed significant allelic effects for QTL_3H_1, QTL_3H_2, and QTL_7H_1 (Fig. 5A-D). No significant allelic effect was detected for the remaining QTL (Supplementary Fig. 3). QTL_3H_1 and QTL_3H_2 were detected in both the BLINK and FarmCPU models, while QTL_7H_1 was associated with two markers, each of which were detected either in the BLINK or FarmCPU models (Table 2).

Following the validation of the QTL detected in our study, we examined the tested genotypes for favorable alleles and analyzed the optimal allelic combinations of the MTAs associated with these QTL. Based on this analysis, we observed 15 allelic combinations, as shown in Supplementary Table S3. Among them, we focused on ten homozygous combinations since they provided a clearer insight into allele effects. One genotype possessed all the favorable alleles and achieved a BLUE value of 3.78. In contrast, 214 genotypes did not contain any favorable allele, and their BLUE values ranged between 32.56 and 51.96. Additionally, 32 genotypes contained a favorable allele from one of the significant MTAs, and their BLUE values ranged from 14.56 to 49.68. Twenty genotypes contained two favorable alleles from either

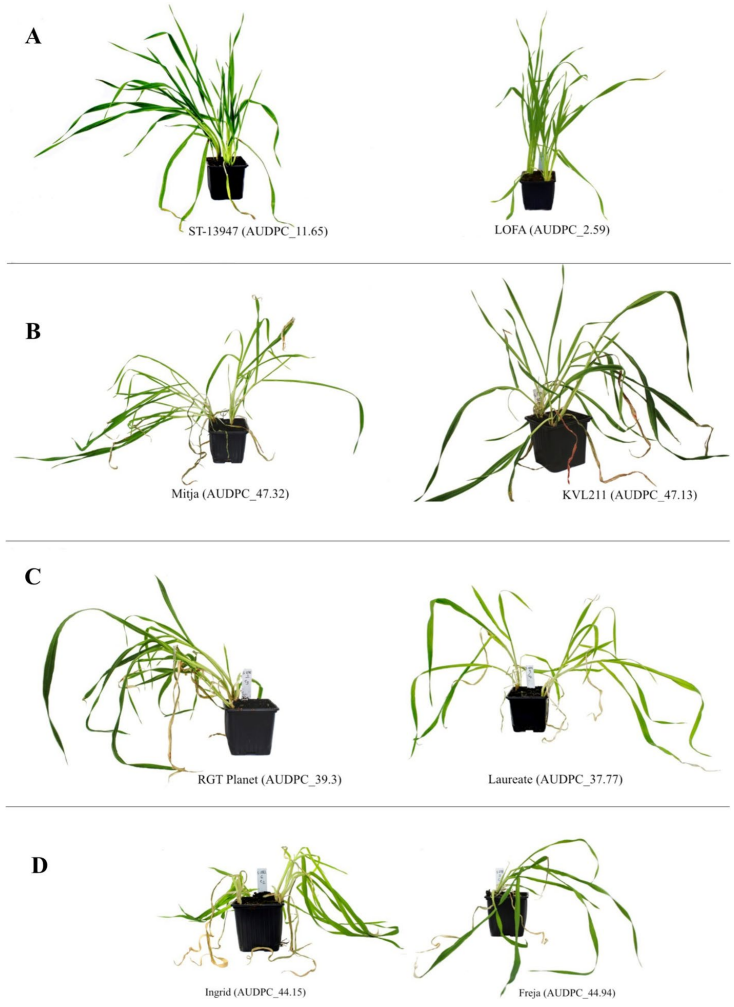


**Fig. 2** Barley genotypes with low or high AUDPC associated with resistance and susceptibility against scald disease and the check cultivars during third scoring (17 DAI). Cultivars having low BLUE values; ST-13947 (left) and Lofa (right) with BLUE of 11.65 and 2.59 respectively; **B** Genotypes having high BLUE values; Mitja (left) and KVL211 (right) with BLUE of 47.32 and 47.13 respectively; **C** Moderately resistant check cultivars; RGT Planet (left) and Laureate (right) with BLUE of 39.3 and 37.77, respectively; **D** Susceptible check cultivars; Ingrid (left) and Freja (right) with BLUE of 44.15 and 44.94 respectively

two of the four significant MTAs, while two genotypes had three favorable alleles, resulting in BLUE values of 10.98 and 27.18, respectively.

## Methods

### Plant materials

A total of 275 spring barley accessions provided by the Nordic gene bank NordGen (https://www.nordgen.org/), along with four commercial check cultivars, were evaluated for scald resistance under greenhouse conditions. The majority of the gene bank germplasms were Swedish landraces. Among the tested cultivars, Freja and Ingrid were susceptible to scald, while Laureate and RGT Planet are known as moderately resistant cultivars. In this study, we conducted two experiments using an augmented block design. In each experiment, the gene bank materials were

**Table 1** Summary of linkage disequilibrium analysis of the 10,151 qualified SNP markers across seven chromosomes and the entire barley genome

| Chromosome | Total number of marker pairs | Avg $r^2$ for total marker pairs | No. of sig. marker pairs and percent of sig. Markers ($p <= 0.001$) * | Avg $r^2$ for sig. pairs ($p <= 0.001$) | LD half-decay (bp) ** |
|---|---|---|---|---|---|
| Chr 1H | 50,879 | 0.15 | 27,721 (54) | 0.15 | 1,449,455 (1.4) |
| Chr 2H | 73,660 | 0.16 | 43,466 (59) | 0.16 | 1,487,502 (1.5) |
| Chr 3H | 79,269 | 0.18 | 48,675 (61) | 0.18 | 2,281,720 (2.3) |
| Chr 4H | 48,522 | 0.17 | 28,066 (58) | 0.17 | 2,751,740 (2.8) |
| Chr 5H | 96,216 | 0.18 | 60,977 (63) | 0.18 | 2,009,142 (2.0) |
| Chr 6H | 71,913 | 0.23 | 48,422 (67) | 0.23 | 4,427,179 (4.4) |
| Chr 7H | 75,867 | 0.17 | 47,473 (63) | 0.17 | 1,835,055 (1.8) |
| Whole genome | 496,319 | 0.21 | 300,613 (61) | 0.21 | 2,083,171 (2.1) |

* Number in brackets represents the percentage of significant marker pairs on each chromosome as well as at the whole-genome level

** Number in brackets represents the LD half-decay in mega base pairs (Mbp)

divided into 14 blocks, each containing a repetition of all four commercial checks and a random subset of the gene bank materials. Four to six seeds per genotype per pot (8 ×8x9 cm in size) were sown in both experiments and were used for disease evaluation.

### Inoculation

The *R. graminicola* pathogen was collected from spring barley fields in Southern Sweden near Ystad in 2022. The specific pathotype of the collected pathogen was unknown. The inoculum was multiplied under in vitro conditions by following an optimized protocol from the Department of Plant Breeding, SLU [31]. The *R. graminicola* mycelia were initially grown on water agar media (15 g of agar per liter of distilled water) for two weeks and then transferred to plates with CZV8 CYM media (Supplementary Table S4). The plates were incubated under dark conditions at 17–18 °C for two weeks until mycelial growth reached 1–3 cm in diameter. The mycelium was then transferred to wheat germ agar media (100 g of wheat germ (Risenta)and 15 g of agar per liter of distilled water). The cultured plates were incubated under dark conditions at 17–18 °C for two weeks to induce *R. graminicola* sporulation. Afterward, the conidia were collected by water flooding the plate and scrapped forcefully with a fine paintbrush. Then, the inoculum suspension was prepared at a concentration of $1.35 \times 10^6$ conidia/ml using tap water and 0.02% of the surfactant Tween® 20. Inoculation was conducted at the third leaf development stage (Zadoks stage 13) by evenly spraying the plant leaves with a hand spray until the plants ran off. Immediately after inoculation, the plants were transferred to an

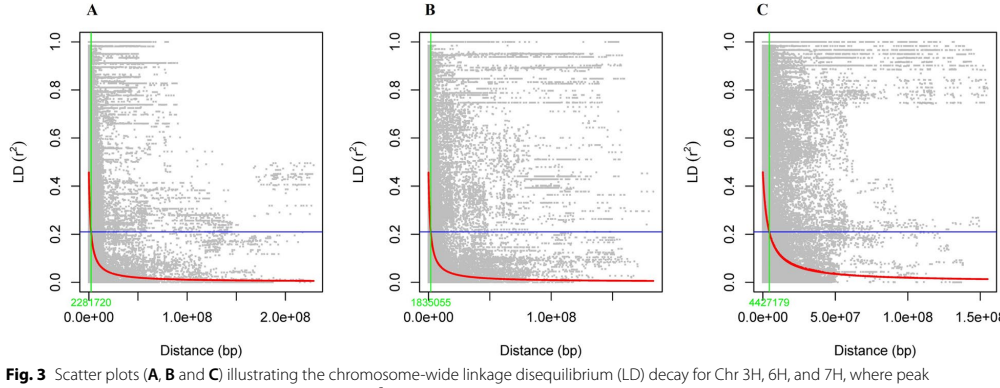


**Fig. 3** Scatter plots (**A**, **B** and **C**) illustrating the chromosome-wide linkage disequilibrium (LD) decay for Chr 3H, 6H, and 7H, where peak markers were identified respectively. The plot displays $r^2$ values against physical distance (in base pairs). The red curve represents the smoothing spline regression model fitted to the LD decay, with a maximum LD decay of 0.46 observed for all chromosomes. A horizontal blue line marks the half-decay $r^2$ value (0.23 for all chromosomes), and a green vertical line indicates the distance between marker pairs (1,181,720 bp, 4,427,179 bp, and 1,835,055 bp for chromosomes 3H, 6H, and 7H, respectively). The intersection between the green line and the blue half-decay line corresponds to the point where LD decay reaches half its maximum value

**Table 2** List of significant SNP markers associated with scald resistance in the tested barley germplasms. Two statistical models, BLINK and FarmCPU, were used to identify eight significant SNP markers

| QTL | SNP marker | Chr | Allele[1] | Pos. (Mbp) | MAF | P. value | Effect | PVE (%) | No. of MTA | Statistical Model |
|---|---|---|---|---|---|---|---|---|---|---|
| QTL_3H_1 | BOPA1_1977-1385 | 3 | G/**A** | 424.23 | 0.05 | 7.05E-09 | 5.65*** | 17.9 | 1 | BLINK |
| | | | | | | 6.42E-08 | 4.99*** | 18.6 | | FarmCPU |
| QTL_3H_2 | JHI-Hv50k-2016–183215 | 3 | A/**G** | 442.21 | 0.133 | 4.75E-09 | −2.91*** | 6.2 | 1 | FarmCPU |
| | | | | | | 2.12E-13 | −4.2*** | 9 | | BLINK |
| QTL_3H_3 | BOPA1_2338-1572 | 3 | G/**A** | 450.44 | 0.108 | 9.68E-08 | −1.67 ns | 0.9 | 1 | FarmCPU |
| QTL_6H | BOPA1_4146-1154 | 6 | **A**/G | 527.21 | 0.281 | 2.85E-06 | 1.65 ns | 0.6 | 1 | FarmCPU |
| QTL_7H_1 | BOPA2_12_20201 | 7 | G/**A** | 4.58 | 0.081 | 5.81E-09 | 4.00*** | 4.9 | 1 | FarmCPU |
| | JHI-Hv50k-2016–441289 | 7 | C/**T** | 5.4 | 0.059 | 3.37E-16 | −6.2*** | 17.3 | 1 | BLINK |
| QTL_7H_2 | JHI-Hv50k-2016–445855 | 7 | T/**G** | 11.1 | 0.065 | 5.32E-07 | −3.7 ns | 6.9 | 1 | BLINK |
| QTL_7H_3 | SCRI_RS_184902 | 7 | G/**A** | 621.55 | 0.197 | 2.25E-06 | −1.89 ns | 2.7 | 1 | FarmCPU |
| | | | | | | 1.38E-08 | −2.46 ns | 3.3 | | BLINK |

*QTL* Quantitative Trait Loci, *Chr* Chromosome, *Pos.* Position, *MAF* Minor Allele Frequency, *PVE* Phenotype Variance Explained (%), *Effect* Allelic effect on the phenotypic variation (the allele listed first represents the major allele, while the allele written in bold signifies the favorable allele)

[1] This is an explanation for the significant difference between favorable and unfavorable alleles over the observed phenotype. ***, $P < 0.0001$; ns, not significant

incubation room with a relative humidity (RH) of 100% and kept under dark conditions for 72 h at 16–17 °C. Three days after inoculation, the growth conditions were adjusted to 75% RH while maintaining a 16/8 h light/dark cycle without altering the temperature.

#### Disease scoring and phenotypic data analysis

Disease evaluation was carried out at 11 days post inoculation (DPI) when the initial symptoms, such as water-soaked lesions, developed on the leaf surface of the inoculated second and third leaves. Subsequent scoring was conducted at 14 and 17 DPI.

The score ranged between 0 and 10, where 0 indicated no disease symptom occurrence, and 10 indicated the highest severity (Fig. 6), following the methods of [32]. The check cultivars were used to adjust the disease scores of the gene bank materials using the AugmentedRCBD package version 0.1.7 in R 4.2.2 [33]. With these adjusted means (disease scores), the area under the disease progression curve (AUDPC) was subsequently calculated using the same statistical package for individual experiments.

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_{i+}y_{(i+1)}}{2}\right)(t_{(i+1)} - t_i)$$

where $y_i$ is the score at the $i^{th}$ observation, $t_i$ is the time (DAI) at the $i^{th}$ observation, and n is the number of observations. The resulting AUDPC values from the two experiments were used to calculate the best linear unbiased estimator (BLUE) using META-R 6.04 software [34].

$$y_{il} = \mu + G_{il} + R_l + \varepsilon_{il}$$

where $y_{il}$ is the AUDPC of the $i^{th}$ genotype in the $l^{th}$ replicate, μ is the general mean value, $G_{il}$ is the $i^{th}$ genotype effect in the $l^{th}$ replicate, $R_l$ is the effect of the $l^{th}$ replicate, and $\varepsilon_{il}$ is the residual effect.

The broad-sense heritability ($H^2$) was calculated using META-R 6.04 software [34].

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

where $H^2$ is the broad-sense heritability and $\sigma_g^2$ and $\sigma_e^2$ indicate the genotype and error variance components, respectively.

#### Genotyping, linkage disequilibrium, and genome-wide association analysis

The tested barley accessions were genotyped using the 15 K Illumina Infinium array from TraitGenetics GmbH (SGS, Germany). Single nucleotide polymorphism (SNP) markers with more than 10% missing values were removed from the analysis, refining the dataset to 13,268 markers, and after setting the minor allele frequency (MAF) threshold at 0.05, a total of 10,151 markers remained for the GWAS. The physical position of the genetic markers were determined following the Morex V3 reference genome [35]. The list of markers and the genotypes used for GWAS are provided in Supplementary Table S5. Two multi-locus models, Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) [36] and FarmCPU (fixed and random model circulating probability unification)

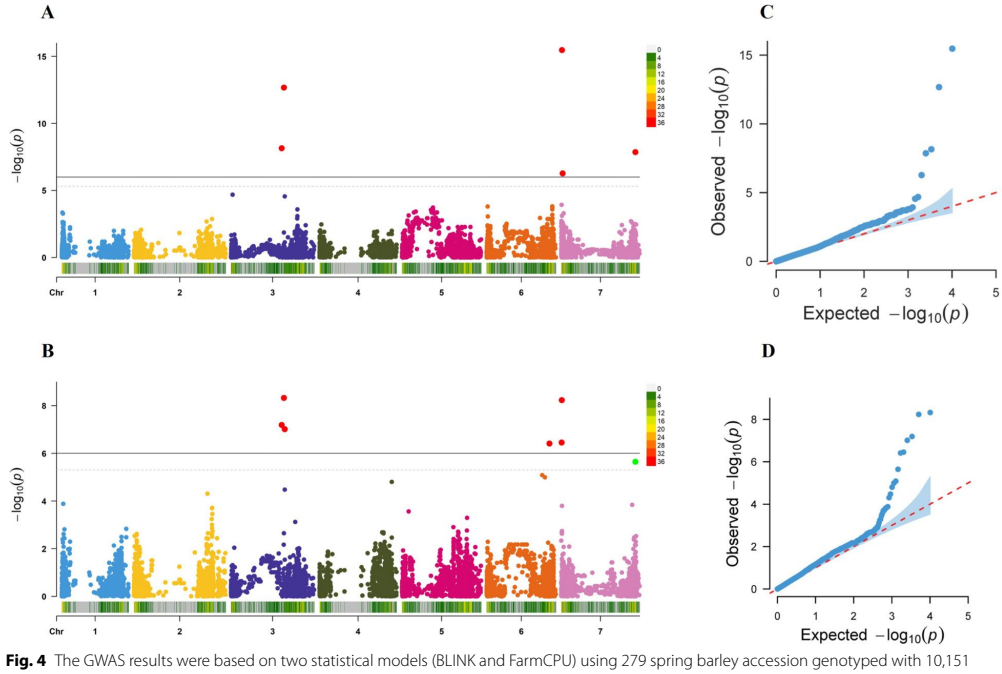


**Fig. 4** The GWAS results were based on two statistical models (BLINK and FarmCPU) using 279 spring barley accession genotyped with 10,151 SNP markers. The Manhattan plots and QQ plots show the marker trait associations from the (**A** and **C**) BLINK and the (**B** and **D**) FarmCPU model. The X-axis of Figs. 5 (**A**) and (**B**) show the chromosomes of barley altogether with the SNP density on each chromosome while the Y-axis represents -log10 (p) values. Again, on the same pictures, the dotted line and block line represented the significance threshold at 0.05 and the Bonferroni correction ($5.0 \times 10e^{-6}$), respectively. Five and six SNPs were detected above the Bonferroni threshold for BLINK and FarmCPU respectively

[37] from GAPIT 3 [38], were used to detect markers associated with scald incidence. The threshold for identifying significant markers was set at a $P$ value $\leq 0.05$ with Bonferroni correction (0.05/number of markers), attaining -log10 (0.05/10,151) $= 5.3$ with a $P$ value of $3.77e^{-6}$.

The squared correlation coefficient ($r^2$) was calculated at the chromosome-wide and genome-wide linkage disequilibrium (LD) levels in TASSEL [39] using a sliding window of 50 SNPs [40]. A total of 10,151 markers were used to estimate LD. The calculated $r^2$ was plotted against the physical distance (i.e., base pairs) between the locus pairs by adding a smoothing spline regression line, and individual chromosome-wide LD decay curves and genome-wide LD decay curves were generated in R 4.2.2 using the genetics package version 0.1.3 [41].

The QTL were identified based on the LD decay, meaning significant detected marker-trait association (MTAs) beyond the chromosome-wide LD decay were considered likely to segregate independently, suggesting different loci influencing the disease resistance [42]. We followed the same strategy for QTL validation. We initially performed a literature search to understand if any QTL reported in previous studies were located within the LD decay distance with the identified QTL in the current study. If we found no QTL, we considered the currently identified QTL a unique one.

#### Favorable allele identification

The allelic effects of the detected MTAs from the GWAS were analyzed to observe their impact on scald resistance. First, a linear model was used with BLUE as the response variable and the detected MTA from the GWAS as the categorical predictor. Then, the least-squares means of the alleles of each MTA were calculated using the emmeans package (version 1.10.1). Subsequently the cld (compact letter display) function was employed in the multcomp package (version 1.4–25) to summarize and interpret the differences in BLUE according to the allele (the results are shown in Supplementary Table S2, S6 and S7). The results were then visualized using the ggplot2 package (version 3.5.1) in R 4.2.2. MTAs with significant

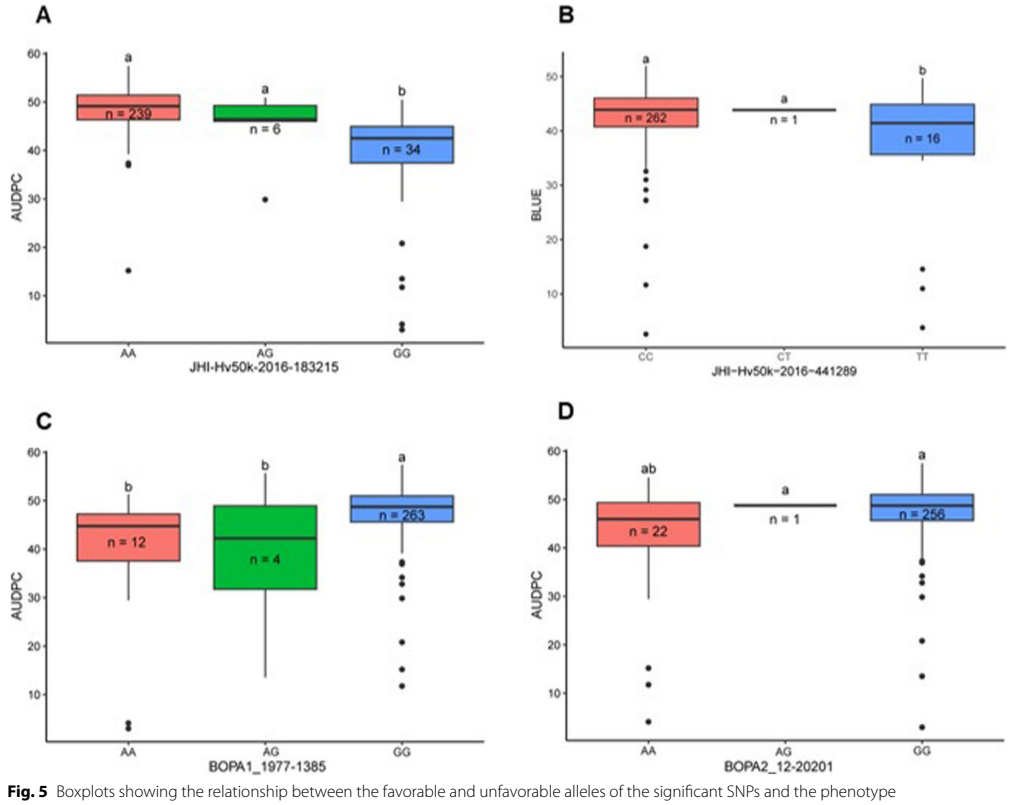


**Fig. 5** Boxplots showing the relationship between the favorable and unfavorable alleles of the significant SNPs and the phenotype of scald resistance (BLUE) in 279 barley genotypes. The impact of the beneficial and non-beneficial alleles of SNPs JHI-Hv50k-2016–183215, JHI-Hv50k-2016–441289 and BOPA1_1977-1385 and BOPA2_12_20201 are visualized in Fig. **A**-**D**. Tukey's HSD (honestly significant difference) test was performed to establish the significance level by comparing the allelic composition of the barley germplasms


**Fig. 6** Scoring scales of *R. graminicola* infection in barley leaf with the increasing order of disease severity, 0 was asymptomatic and 10 was the highest severity of infection

allelic effects were selected, and their combination effects on the BLUE values were observed.

## Discussion

This study analyzed the resistance of 275 spring barley germplasm with four check cultivars that had different levels of scald resistance using phenotypic data collected under greenhouse conditions. The analysis revealed a significant phenotypic variation among the tested germplasms and a heritability estimate of 0.73.

In addition to heritability, linkage disequilibrium (LD) analysis helps the breeders identify the genomic regions linked to plant disease resistance more accurately [43]. The current study identified the LD decay at the chromosome and whole genome levels (1.4–4.4 Mbp across seven chromosomes and 2.1 Mbp for the whole genome) using 10,151 SNP markers. Many studies have reported a different range of LD decay associated with barley scald disease, each associated with different SNP densities. For instance, an association mapping study using 316 spring barley genotypes reported the LD decay of ~600 kb while using 36,793 SNP markers and identified 15 QTL associated with seedling stage scald resistance [44]. Similarly, another association study with a barley core collection (298 elites and 812 plant genetic resources) observed an LD decay of 2 Mbp while using 57 million SNP markers and identified 22 QTL [45]. Interestingly, some studies used much higher SNP densities and reported a higher LD decay. For example, a barley MAGIC population study using 27,407 SNP markers reported an LD decay ranging from 7 to 19 Mbp across seven chromosomes, detecting six QTL associated with scald resistance [46]. A shorter LD decay is beneficial because it enhances the precision of genomic regions associated with the trait and assists in developing a more efficient breeding strategy [47]. Even with a lower SNP density, the LD decay in our study captured important genetic variations, highlighting the efficiency of our approach in identifying important genomic regions associated with scald resistance.

Among the three QTL identified on chromosome 3H, QTL_3H_1 is likely a potential new QTL identified for scald resistance. This QTL is located at 424.23 Mbp on Chr 3H, and the nearest QTL previously identified is QTLR3H.4 (428.8 Mbp). This QTL was identified as a scald-resistant QTL in double haploid (DH) mapping populations of winter barley (a population developed between Saffron, the susceptible cultivar, and Retriever, the cultivar resistant to scald) The author considered that QTLR3H.4 overlapped with the *Rrs1* locus, affecting 30% of the phenotypic variance of their tested barley population. The distance (4.6 Mbp) between QTLR3H.4 and QTL_3H_1 from the current study exceeds the observed LD decay (2.3 Mbp) at Chr 3H, suggesting that the linkage between these QTL is not strong enough and has a chance of being separated during segregation. Another QTL, QTL_3H_2, was found only 490 kb away from *Rrs. B87 was* identified in the B87/14 spring barley line [48]. T The author considered that QTLR3H.4 overlapped with the *Rrs1* locus, affecting 30% of the phenotypic variance of their tested barley population. The distance (4.6 Mbp) between QTLR3H.4 and QTL_3H_1 from the current study exceeds the observed LD decay (2.3 Mbp) at Chr 3H, suggesting that the linkage between these QTL is not strong enough and has a chance of being separated during segregation. Another QTL, QTL_3H_2, was found only 490 kb away from *Rrs.B87 was* identified in the B87/14 spring barley line [49]. This gene is considered a single dominant gene against *R. graminicola* located close to the well-known *Rrs1* complex locus [49]. Avr-*Rrs1* triggers the resistance mechanism by recognizing the necrosis-inducing protein 1 (NIP1) fungal protein via a resistant host plant carrying the *Rrs1* gene [50]. Additionally, it was reported that *Rrs1* suppresses the growth of fungal hyphae and leads to the formation of a fungal network that grows randomly in different directions instead of forming a functional fungal network in Atlas 46, a resistant cultivar that possesses the *Rrs1* gene [51]. Another QTL, QTL_3H_3 (450.44 Mbp), located at 449.9 Mbp, was found to be proximate to QTLCB3H.4, which was previously identified in the Spanish landrace-derived line SBCC145 [52]. In addition, several QTL contributing to scald resistance have been detected on Chr 3H in various spring barley collections. These genes are located at 447.3 Mbp, 454.9 Mbp, and 455.3 Mbp on Chr 3H [10, 11, 52–54]. Possible explanations for the presence of multiple QTL related to scald resistance along Chr 3H have been proposed as follows: (1) *Rrs1* is a complex locus containing many alleles of the same R gene; (2) *Rrs1* is a set of closely connected genes; or (3) *Rrs1* is a combination of both [11].

Another significant QTL detected in the current study is QTL_6H, which is also considered a new QTL for scald resistance. Studies have identified 30 QTL related to barley scald resistance on Chr 6H with a total physical distance equivalent to 580.00 Mbp [11, 16, 20, 21, 48, 54–59]. Among these QTL, Qsc3.6H.7-Seebe (545.5 Mbp) [54], is the closest QTL to QTL_6H detected in the present study, despite the considerable distance of 34.8 Mbp between them (510.7 Mbp vs. 545.5 Mbp). Notably, most of these QTL (25 out of 30 QTL) were detected at the proximal region of Chr 6H, while only 5 QTL were identified at the distal end of the chromosome. Although the location of the QTL, whether proximal or distal, may not directly impact the phenotype, identifying new QTL at the distal end where the QTL has been sparse contributes

to the diversification of genetic factors known to influence scald resistance in barley.

Four MTAs were detected on Chr 7H via GWAS, and three QTL were identified. Among the four MTAs, two (BOPA2_2_20201 and JHI-Hv50k-2016–441289) were located close to each other (only 820 kilobase pairs apart) and therefore considered the same QTL (QTL_7H_1). At the same time, this QTL was found to be near the previously known QTL *Rrs2*. One of the earliest reports related to *Rrs2* [60] reported that in Digger (the resistant cultivar), halos were formed in the cell walls, and larger papillae were produced than in Osiris (the susceptible cultivar), despite the identification of the causal gene mediating such subcuticular modification. Later, *Rrs2* was fine-mapped in the $F_2$ population derived from a cross between Atlas (the resistant cultivar) and Steffi (the susceptible cultivar), and the physical position of *Rrs2* was detected at 5.2 Mbp [13]. This *Rrs2* gene overlaps with QTL_7H_1 from our study.

QTL_7H_2 was closely located to the previously reported QTL, *Rh2*, which is located at position 10.8, and this QTL was detected in the Atlas, a scald-resistant spring barley cultivar [61]. To our understanding, QTL related to other major barley diseases are also located at the end of the 7H short arm region, such as Rdg2a for barley leaf stripe [62] and Rpg1 for barley stem rust [63] near QTLTritonRrs7H271, making this region of Chr 7H worthy of further evaluation to identify potential scald-resistant loci. Furthermore, QTL_7H_3 is a new locus identified in the current study, and it is located at 621.6 Mbp on Chr 7H. Its nearest known QTL is *Rrs15* (626.3 Mbp) [18], which is located at a distance of 4.7 Mbp from QTL_7H_3. *Rrs15*, derived from the Israeli accession of wild barley, was successfully mapped in the third backcross population between that accession and a scald-susceptible cultivar called Clipper [18].

Two genotypes, AKKA and FRIDA possessed three favorable alleles each. However, the presence of an unfavorable allele of one of the MTAs detected at QTL_7H_1 (relevant MTA: JHI-Hv50k-2016–441289) in FRIDA reduced resistance, resulting in a higher BLUE value. In contrast, for AKKA, the favorable alleles from both MTAs at QTL_7H_1 likely worked synergistically to enhance resistance, even without the favorable allele from QTL_3H_1. Remarkably, the favorable alleles of QTL_3H_1 and QTL_7H_1 (relevant MTA: JHI-Hv50k-2016–441289) were found together only in the genotype Solar, which possesses all the favorable alleles. A study reported that the presence of favorable alleles across different QTL can enhance resistance. However, the combinations of all these QTL may rarely occur in different genetic backgrounds [64]. Twenty genotypes with two favorable alleles from either two of the significant MTAs showed further decrease in resistance, with an average BLUE of 34.61. Based on the results from haplotype comparisons (Supplementary Table S3), the BLUE values of these haplotypes were not statistically different. They are still more resistant than those with a single allele (with an average BLUE of 42.05) and those without any favorable allele (with an average BLUE of 44.1). Thirty-two genotypes had a single favorable allele from one of the significant MTAs. Following the results from the haplotype comparisons, there was no significant difference between the average BLUE values of the genotypes with one favorable allele and those with no favorable allele. However, when examining the individual genotype, two genotypes (MENTOR and KORU) with only one favorable allele from the MTA, BOPA1_1977-1385 had higher BLUE values than the susceptible checks with no favorable allele. The only favorable allele presented in MENTOR and KORU was located at the QTL_3H_1 region near the *Rrs1* complex locus. However, this QTL alone could not overcome the unfavorable alleles'strong collective effect. Another possibility is that these unfavorable alleles interact with other unfavorable alleles in other genomic regions, dominating the favorable allele effect.

In summary, identifying new QTL enhances our understanding of scald resistance in barley and highlights potential targets for future breeding programs. The discovery of novel loci and their relationships to known resistance genes provides valuable insights into the genetic architecture of disease resistance, which could be leveraged to develop more resilient barley cultivars. Research focusing on fine-mapping candidate genes in these detected QTL regions should be conducted to identify the causal variants for scald resistance. While these types of genetic variants can be discovered and evaluated under controlled environmental conditions, field experiments should be carried out not only considering the effects of environmental conditions and their interactions but also considering the stability of the effects of QTL across both space and time, thereby strengthening the resulting breeding strategies that were identified in this research.

## Conclusions

The current study utilized association mapping analysis to examine the genetic variation associated with scald disease in 279 spring barley genotypes. The significant genetic variation observed among the barley germplasms highlights the abundant genetic diversity within the tested gene bank germplasm. This diversity is valuable for improving crop performance and resilience in breeding programs. Furthermore, our study revealed three novel QTL that offer fresh insights into the genetic basis

of resistance traits and three previously identified QTL, reinforcing the robustness and reliability of this investigation. Additionally, MTAs with favorable effects on disease progression could be valuable for enhancing barley scald resistance through marker-assisted breeding targeting the development of barley varieties with strong resistance, underscoring the importance of ongoing research programs. Additionally, future studies, such as identifying candidate genes within these QTL and developing KASP markers for breeding programs targeting improving scald resistance and enhancing crop productivity through precision breeding strategies, are suggested.

## Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Not applicable.

**Authors' contributions**
AC and MZ conceived and designed the study. SMN and MZ conducted the greenhouse experiments and performed scald disease evaluation at seedling stage. SMN and JÅ analyzed data and wrote the first draft. AC, EJ, PKS and JÅ supervised and reviewed the manuscript. All authors approved the final version of this manuscript.

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**Data availability**
The data that support the findings of this study are provided in the additional files.

## Declarations

**Ethics approval and consent to participate**
This article does not contain any studies with human participants or animals. The collection materials of the plants complies the relevant institutional, national, and international guidelines and legislation.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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## Publisher's Note

# III

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# Comparing wMAS, GWAS, and genomic prediction for selecting powdery mildew-resistant spring barley genotypes

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## Abstract

**Background** Barley is one of the most widely cultivated cereals worldwide, and powdery mildew is among the major diseases threatening global barley production. Our study evaluated 370 spring barley breeding lines under controlled greenhouse growth conditions.

**Results** Using genome-wide association study (GWAS), 21 quantitative trait loci (QTL) were identified associated with seedling-stage powdery mildew resistance. Of these, eight were newly identified in this study. Genetic merit was also calculated using major-effect markers, and a positive correlation (> 0.7) was observed between the genetic merit and BLUP (AUDPC) values in both the two subpopulations of two- and six-row barley. While evaluating the performance of genomic prediction (GP) models, a GWAS-incorporated GP model consistently outperformed the Standard GP model in both subpopulations demonstrating the advantage of incorporating major-effect markers for a more accurate prediction. Our analysis of genotype selection patterns revealed a notable degree of agreement among the tested methods. In the two-row subpopulation, a large number of genotypes were exclusively selected by weighted marker-assisted selection (wMAS) revealing the dominance of major-effect QTL. In contrast, the six-row subpopulation had a smaller wMAS-exclusive group, suggesting a more polygenic background, which was captured by genomic prediction. Additionally, genomics-based methods consistently identified resistant genotypes that were overlooked by phenotypic selection, showing their ability to detect hidden genetic potential.

**Conclusions** Overall, GWAS-incorporated GP model demonstrated the best performance among the evaluated methods, suggesting this approach is the most effective with a potential to contribute to efficient breeding of powdery mildew resistance in spring barley.

**Keywords** GWAS, wMAS; genomic prediction, Barley, Powdery mildew, rrBLUP

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## Background

Barley (*Hordeum vulgare* L.) is a domesticated crop with a long history originating in the Fertile Crescent [1]. Due to its resilience to unfavorable climate conditions such as drought and poor soil fertility, it is considered an important cereal crop [2]. Barley is the fourth-largest crop globally, with an estimated production of 145 million tons in 2023.

Powdery mildew (PM), caused by the biotrophic fungus *Blumeria graminis* f. sp. *Hordei (Bgh)*, is a major disease on barley affecting production worldwide, although in particular in temperate regions. *Bgh* causes white patches on the barley leaves and stems, which disturb photosynthesis and reduce yield [3]. Disease management in barley production relies on both fungicides and resistant varieties, though genetic resistance remains the most sustainable solution [4]. Traditionally, phenotypic evaluation has been the primary method to select superior genotypes, although such methodologies are time-consuming and often require several generations of the crop for a proper selection. The dawn of genomics tools facilitated the first QTL mapping through linkage analysis, even though these approaches heavily relied on pedigree along with phenotypic information [5].

More than 30 distinct PM resistance genes have been identified in barley, providing important information for breeding resistant cultivars [6, 7]. Major resistance genes such as *Mla1*, *Mla6*, *Mla12*, *Mlg*, *Mlp*, *Mlk*, and *Mlh* provide race-specific resistance to distinct *Bgh* isolates [8]. Another well-known PM-resistant gene is *mlo*, whose resistance is mediated by a recessive mechanism. Unlike the race-specific resistant genes (major genes) described above, *mlo* provides a broad-spectrum durable resistance and inhibits pathogen growth at the earliest stage of infection [6]. Recent developments such as genome-wide association study (GWAS) provides significant advantages over QTL mapping by using a greater allelic diversity [9]. However, both QTL mapping and association mapping are still useful tools for breeding programs by enabling the exploration of QTL associated with various traits.

Recently, genomic prediction (GP) has increased in popularity as this methodology is predicting the breeding values of individuals based on their genomic information without their phenotypic data [10]. A GP model is trained by using a specific training population with known genotypic and phenotypic data, and thereafter, the genomic estimated breeding values (GEBVs) of an independent population can potentially be calculated without prior phenotypic data. The predictive ability (PA) of the model is measured by correlating the predicted values with the observed values [11]. Many factors can influence the PA including the size of training population, quality of phenotypic data and trait complexity [10].

GP studies utilize models, including ridge regression best linear unbiased prediction (rrBLUP) and Bayesian models, both of which treat all markers as random effects [12]. Additionally, models combining random- and fixed-effects has also been employed [13]. Each of the models are employing a different algorithm, contributing to the challenges of determining which model is preferable to use [14, 15]. Thus, in a study aimed to predict seedling stage resistance against Septoria Tritici Blotch in mixed winter wheat cultivars, the GP model, incorporating GWAS-significant markers as fixed effects, resulted in more significant predictions than rrBLUP, which used all markers as random effects [16]. However, the rrBLUP model was superior to the GWAS-incorporated GP model for the predicting of spot blotch resistance in bread wheat breeding populations [13].

With the increasing application of genomic tools in plant breeding, a comprehensive and direct comparison of different genotype selection methods are becoming important. Such a comparison should include traditional phenotypic selection (PS), weighted marker-assisted selection (wMAS), and genomic selection (GS), since each of these methods have its own advantage for capturing the underlying genetic variation, which is then optimized in breeding strategies [17, 18]. Due to the significant effects of powdery mildew on barley, it is strategically important to identify the most effective and efficient selection strategies to improve resistance, and to make targeted decisions in practical plant breeding.

This study evaluated 370 advanced spring barley breeding lines developed by Lantmännen AB, Sweden, with the following aims: (1) to identify QTL associated with seedling stage powdery mildew resistance in spring barley using GWAS (2) to develop and evaluate GP models for powdery mildew resistance considering different training populations and marker sets (3) to evaluate four selection methods: PS, wMAS, and two GS strategies, i.e. a Standard GP approach, and a GWAS-incorporated GP approach for identifying superior breeding lines for powdery mildew resistance.

## Methods

### Plant materials and growth conditions

A total of 370 advanced spring barley breeding lines (219 lines for two-row and 151 lines for six-row type) were provided by Lantmännen and tested for powdery mildew resistance under controlled conditions at Svalöv, Sweden, in 2022 and 2023. Two commercial cultivars, Anneli (two-row cultivar) and Judit (six-row cultivar), were included as checks in both experiments. All these materials were randomly arranged in an Alpha Lattice design with three replicates and the whole experiment was repeated twice. Approximately ten seeds per genotype were grown with $10 \times 10$ cm spacing in the aluminum

benches in the greenhouse. Seedlings were watered with a sprinkler once a day, and the light condition was maintained at a 16-hour light/8-hour dark cycle.

#### Inoculation and disease evaluation

Powdery mildew-infected leaves were collected from the barley growing fields in Svalöv. Inoculation relied on field-collected powdery mildew samples, and the *Bgh* isolates present were not characterized. Being an obligate pathogen, *Bgh* was grown on live barley plants using the cultivars Anneli and Judit. One day before inoculation, the old conidia were removed by shaking them off from the cultured plants to get fresh and infectious inoculum. The inoculation was carried out twice, 9 days and 11 days after germination, by directly brushing infected plants against the uninfected test plants in both experiments. The growing chamber was maintained at constant light/dark and humidity conditions, as mentioned in the plant materials and growth conditions section. Disease scoring was performed three times (11, 13, and 15 days post inoculation, DPI) during the first experiment and four times (14, 16, 18, and 20 DPI) during the second experiment. This difference in scoring timing and frequency was adjusted based on the observed differences in disease progression rate between the two experiments. The disease severity was assessed at the whole-plot level by assigning a single mean score on a 0–4 scale (5 categories, of which 0 represents no infection and 4 represents the highest infection, ≈ 100% severity) [6]. (Fig. 1).

Disease scores collected at each time point were adjusted for the partially balanced incomplete design (PBIB) using PBIB.test function from the Agricolae package following the statistical model [19, 20].

$$Y_{ilm} = \mu + G_{ilm} + B_{lm} + R_m + \varepsilon_{ilm}$$

where: $Y_{ilm}$ is the score of $i^{th}$ genotype in the $l^{th}$ block at the $m^{th}$ replicate; μ is the overall mean; $G_{ilm}$ is the effect of $i^{th}$ genotype in the $l^{th}$ block at the $m^{th}$ replicate; $B_{lm}$ is the block effect at $m^{th}$ replicate; $R_m$ is the $m^{th}$ replicate and $\varepsilon_{ilm}$ is the residual error.

These adjusted means were used to calculate area under disease progress curve (AUDPC) [21].

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

, where yi is the score at ith observation; ti is the time (DPI) at the ith observation and n is the number of observations (genotypes).

Finally, best linear unbiased prediction (BLUP) was calculated for each genotype using the AUDPC values from the two experiments with the following statistical model in META-R software [22].

$$Y_{il} = \mu + \mathrm{Gen}_i + \mathrm{Rep}_l + \varepsilon_{il}$$

, where Yil is the AUDPC of the ith genotype in the lth replicate (experiment), μ is the overall mean, Gil is the ith genotype effect in the lth replicate (experiment) and εil is the residual effect.


**Fig. 1** Powdery mildew disease scales ranging from 0 to 4 are used in current experiment. 0 represents the least infection (with almost no infection), and the infection increases as the number increases, reaching a maximum of almost 100% at score 4. Scoring was done by each genotype row by using the scoring categories in this figure. (Blue shading appeared on some leaves is due to the reflection of adhesive tape used during imaging and does not represent a biological feature.)

#### Marker information and quality control

The tested breeding materials were genotyped with 15 K Illumina Infinium array from TraitGenetics GmbH (SGS, Germany) (https://sgs-institut-fresenius.de/en/gesundheit-und-ernaehrung/traitgenetics). Marker information, including physical position, chromosome, and reference genome, was retrieved from the Triticeae Toolbox (T3) [23] and BARLEYMAP [24]. Markers with more than 10% missing data and those with minor allele frequency (MAF) of less than 5% were removed using TASSEL software version 5.2.93 [25]. After filtering, 8,088 SNPs for two-row subpopulation and 6,915 SNPs for six-row subpopulation remained for GWAS analysis.

#### Genome-wide association study (GWAS)

GWAS was performed for two-row and six-row subpopulations separately to minimize the cofounding effects related to the population structure and maximize the identification of population-specific markers, avoiding the masking effect of a combined analysis [26–28], using BLINK [29] model from the GAPIT package (version 3.4) in R 4.3.3 [30]. Significantly associated markers were identified by setting the False Discovery Rate (FDR) at

0.05 and applying the Bonferroni threshold (0.05 divided by the number of filtered SNPs). The Bonferroni thresholds were 6.18 $e^{-6}$ and 7.23 $e^{-6}$ for two-row and six-row subpopulations, respectively.

To characterize the extent of linkage disequilibrium (LD) across each chromosome, pairwise LD ($r^2$) between all SNP markers was calculated in TASSEL [25] with a sliding windows 50 SNPs [31]. The LD ($r^2$) values were plotted against physical distance (Mbp) for each chromosome. Chromosome-wide LD decay plots are presented in Supplementary Figure S1 and S2. For QTL identification, marker-trait associations (MTAs) within the LD decay distance were considered to represent the same locus [32].

#### Weighted marker-assisted selection within spring barley subpopulations

GWAS was performed for each subpopulation as described above, using 6,915 markers for the six-row and 8,088 markers for the two-row populations. For the two-row population, only one significant marker was detected for powdery mildew resistance; therefore, wMAS for this group relied on genotype classes at this single locus. The genetic contribution of a marker was estimated by multiplying the marker's effect size (i.e., additive effect obtained from GWAS results) by the genotype's allele composition (0 = no favorable allele, 1 = one favorable allele, or 2 = two favorable alleles). The cumulative genetic values were predicted by summing the contributions of individual markers according to the following equation [33].

$$Y = \mu + \sum_{i=1}^{n} a_i b_i + \varepsilon$$

Where: Y is the predicted genetic value; μ is the overall mean; $a_i$ is the estimated additive effect of the $i^{th}$ marker; $b_i$ is the genotype's allele composition at the $i_{th}$ marker; ε is the residual.

Genotypes with the lowest predicted genetic values were considered most resistant, following the assumption that lower BLUP (AUDPC) values reflect reduced disease severity, as aligned with the wMAS selection criterion used in this study.

#### Genomic prediction using standard and GWAS-incorporated models

Beyond the direct application of major QTL in wMAS, we also investigated the potential of genomic prediction to improve the breeding efficiency of powdery mildew resistance in spring barley. Using filtered genotypic data, GP analyses were primarily performed for each subpopulation using mixed.solve function from rrBLUP package (version 4.6.3), based on the following linear mixed model [34]:

$$Y = X\beta + Z\mu + \varepsilon$$

where: Y is the vector of adjusted BLUP (AUDPC) values; X and β are the design matrix and vector of fixed effects; Z and μ are the design matrix and vector for random effects (i.e., filtered SNP markers which varied by population); ε is the residual.

Each tested population was randomly divided into a training set (80%) and a validation set (20%). Two main GP strategies were applied.

*GWAS_FIX_rrBLUP*: This model included Bonferroni-significant markers identified via GWAS as fixed effects, while the remaining markers were treated as random effects. GWAS was performed within each training set independently. The significant markers from each run were then incorporated into the prediction model. This strategy was repeated 20 times, and PA was recorded for each run.

*STD_rrBLUP*: In this model, no marker was preselected; all markers were included as random effects. This model was repeated 500 times, and PAs were calculated.

#### Genotype selection: phenotypic, genotypic, and weighted marker-assisted selection

Afterward, we evaluated PS, GS, and wMAS to determine which genotypes were selected or discarded by one or more of the methods. Due to random splitting of the population, genotypes may appear multiple times in the validation sets. Therefore, the mean GEBV for each genotype was calculated by averaging across all its validation appearances. Based on the average GEBVs, two selection thresholds were defined: the top 10% and 20% of genotypes with the lowest disease incidence, i.e., the lowest BLUP for PS and the lowest GEBVs for GS, and the lowest genetic merit for wMAS. However, unlike other selection methods that used GEBVs and BLUPs to define top percentiles, wMAS in the two-row subpopulation relied on discrete marker scores (genetic merit) derived from a single significant GWAS marker, with only genotypes carrying the favorable allele being selected.

## Results

#### Selection method 1: phenotypic evaluation of powdery mildew resistance

The ANOVA revealed significant phenotypic differences in BLUP (AUDPC) values among genotypes, indicating variation in resistance to powdery mildew (Table 1). BLUP (AUDPC) values ranged from 2.43 to 17.38, and

**Table 1** Analysis of variance (ANOVA) for PM resistance from 370 commercial barley breeding lines together with two commercial barley cultivars recorded from two experiments

| Analysis | Population | |
|---|---|---|
| | two-row (2r) | six-row (6r) |
| Minimum BLUP | 2.43 | 2.47 |
| Maximum BLUP | 17.29 | 17.38 |
| Mean | 11.47 | 12.1 |
| Heritability | 0.89 | 0.85 |
| CV | 22.98 | 18.59 |
| Genotype significance | 8.5E-51 | 1E-28 |

their distribution is shown in Fig. 2. The check cultivar, Judit (two-row), exhibited the highest BLUP (AUDPC) value of 17.38 while the other check cultivar, Anneli (six-row), attained 14.45. The broad-sense heritability was above 0.80 for both subpopulations. The calculated BLUP (AUDPC) values were directly used for PS by ranking the genotypes in ascending order at different selection thresholds based on their observed resistance performance (Supplementary Table S1 and S2).

### Selection method 2: association between genetic merit and phenotypic values

The genetic merit was calculated for each genotype using the allelic effects of significant markers, for both the two-row and six-row subpopulations individually. A Pearson correlation was calculated between genetic merit and BLUP (AUDPC) values, resulting in a correlation of 0.76 for two-row and 0.73 for six-row, which indicates that a significant proportion of phenotypic variation was captured.

#### *Genome-wide association analysis for powdery mildew resistance*

GWAS was conducted independently for the two-row and six-row spring barley subpopulations, as described in the Methods section, and by this, three markers (using 6,915 markers) for six-row and one marker for two-row

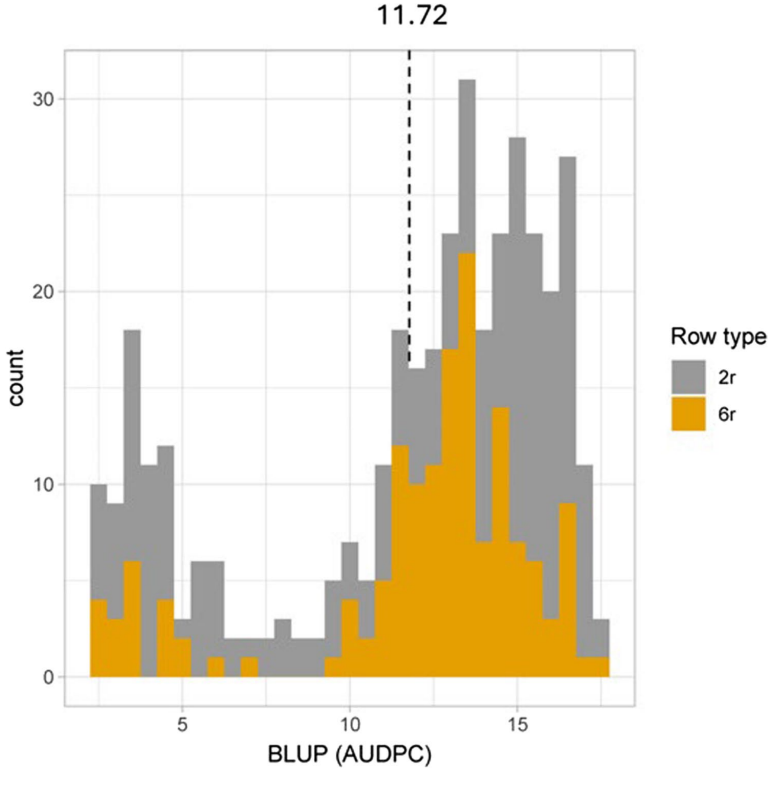


**Fig. 2** Distribution of best linear unbiased prediction (BLUP) values for powdery mildew disease among the tested spring barley breeding lines. The grey-colored bar represents the distribution of two-row barley (219 genotypes, including the check), referred to as 2r in the legend. The yellow-colored bar represents six-row barley (151 genotypes, including check), referred to as 6r in the figure. The mean BLUP (AUDPC) of the entire population was 11.72

**Table 2** List of significant MTAs for powdery mildew resistance detected in two-row and six-row spring barley subpopulations

| SNP | Chr | Pos. (Mbp) | *P* value | Effect | MAF | alleles* | PVE (%) | detected population |
|---|---|---|---|---|---|---|---|---|
| JHI_Hv50k_2016_4209 | 1 | 4.51 | 2.74E-10 | -1.22 | 0.229 | **A**/G | 8.40 | six-row |
| JHI_Hv50k_2016_266364 | 4 | 589.09 | 4.74E-24 | -3.11 | 0.199 | C/**T** | 50.57 | six-row |
| JHI_Hv50k_2016_321026 | 5 | 498.03 | 2.33E-06 | -1.13 | 0.137 | **G**/T | 6.13 | six-row |
| JHI_Hv50k_2016_266643 | 4 | 589.33 | 1.00E-35 | -3.25 | 0.355 | **C**/T | 61.15 | two-row |

*Chr* Chromosome, *Pos* Position in Mega base pair (Mbp), *MAF* Minor Allele Frequency; Effect, Allelic effect (*the allele written in bold represents the favorable allele; *PVE* Phenotype Variance Explained by the detected marker (%)

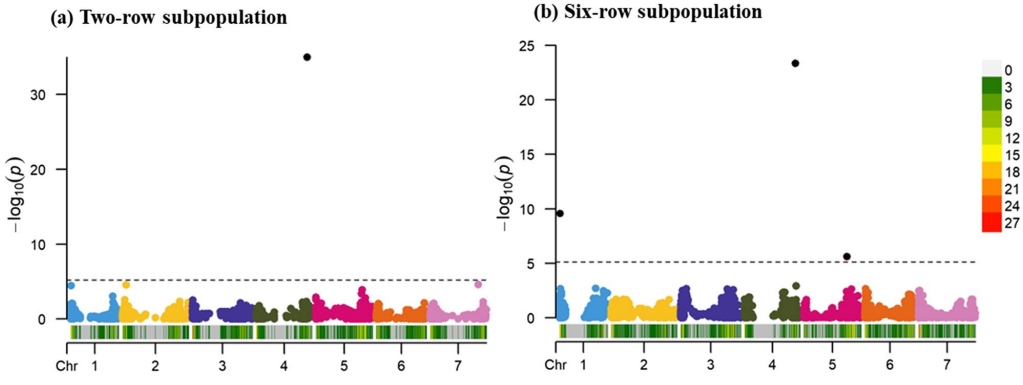


**Fig. 3** Manhattan plot showing marker-trait associations for powdery mildew resistance in the (**a**) two-row and (**b**) six-row barley subpopulations. Each point represents SNP marker plotted against its genomic position and $-\log_{10}(p)$ value. The dashed line indicates the Bonferroni significance threshold. The color-coded bar below the X-axis shows SNP density across the genome, with the legend indicating density levels from low (white) to high (red)

(using 8,088 markers), significant at the Bonferroni threshold, were obtained (Table 2). The Manhattan plots for these two GWAS analyses are illustrated in Fig. 3.

The MTAs to the detected markers were in the six-row subpopulation; JHI-Hv50k-2016-4209 on Chr 1 H (at 4.51 Mbp, explaining 8.40% PVE), JHI-Hv50k-2016-266364 on Chr 4 H located at 589.09 Mbp, and this MTA exhibited a substantially high PVE of 50.57%, and JHI-Hv50k-2016-321026 on Chr 5 H presented at 498.03 Mbp, with 6.13% PVE. The single MTA identified in the two-row subpopulation, JHI-Hv50k-2016-266643, was located at the distal end of Chr 4 H (589.33 Mbp), and accounted for 61.15% PVE, which demonstrated its large effect on the powdery mildew resistance phenotype. The significant MTAs are summarized in Table 2, and in this study, the ranking and selection of genotypes for powdery mildew resistance were based on these MTAs together with their estimated allelic effects, which were later used to calculate genetic merit.

## Selection method 3 and 4: Genomic prediction Model performance

### *Marker identification and testing their consistency for GWAS-incorporated GP model*

A total of 39 unique MTAs were detected, and among them, two MTAs were shared between the two subpopulations. The marker JHI-Hv50k-2016-266643 was most consistently found in the two-row population, appearing in 17 out of 20 runs. SCRI_RS_60293 showed up 8 times, and SCRI_RS_174063 appears four times. Two other markers showed up three times, while the rest were detected only once. In the six-row population, JHI_Hv50k_2016_266496 and JHI_Hv50k_2016_266192 appeared in each of the six runs. JHI_Hv50k_2016_266364 was found in four runs while SCRI_RS_60145 and JHI_Hv50k_2016_321991 were in three runs. The remaining markers were observed at maximum twice (Fig. 4 and Supplementary Table S3).

### *Predictive ability across the GWAS-incorporated and standard GP models*

The predictive abilities (PAs) of the tested GP models across different iterations are illustrated in Fig. 5, and the detailed results are provided in Supplementary Table S4 to S7.

The GWAS-incorporated GP (GWAS_FIX_rrBLUP) model showed a higher average PA than the standard GP (STD_rrBLUP) model in both subpopulations. For the two-row subpopulation (Fig. 5a, top left panel), the average PA was 0.77, with the PA fluctuating between 0.55 and 0.89 across 20 iterations. For the six-row subpopulation (Fig. 5a, bottom left panel), the GWAS-incorporated GP model achieved a comparable average PA of 0.79, with a range of 0.65 to 0.95.

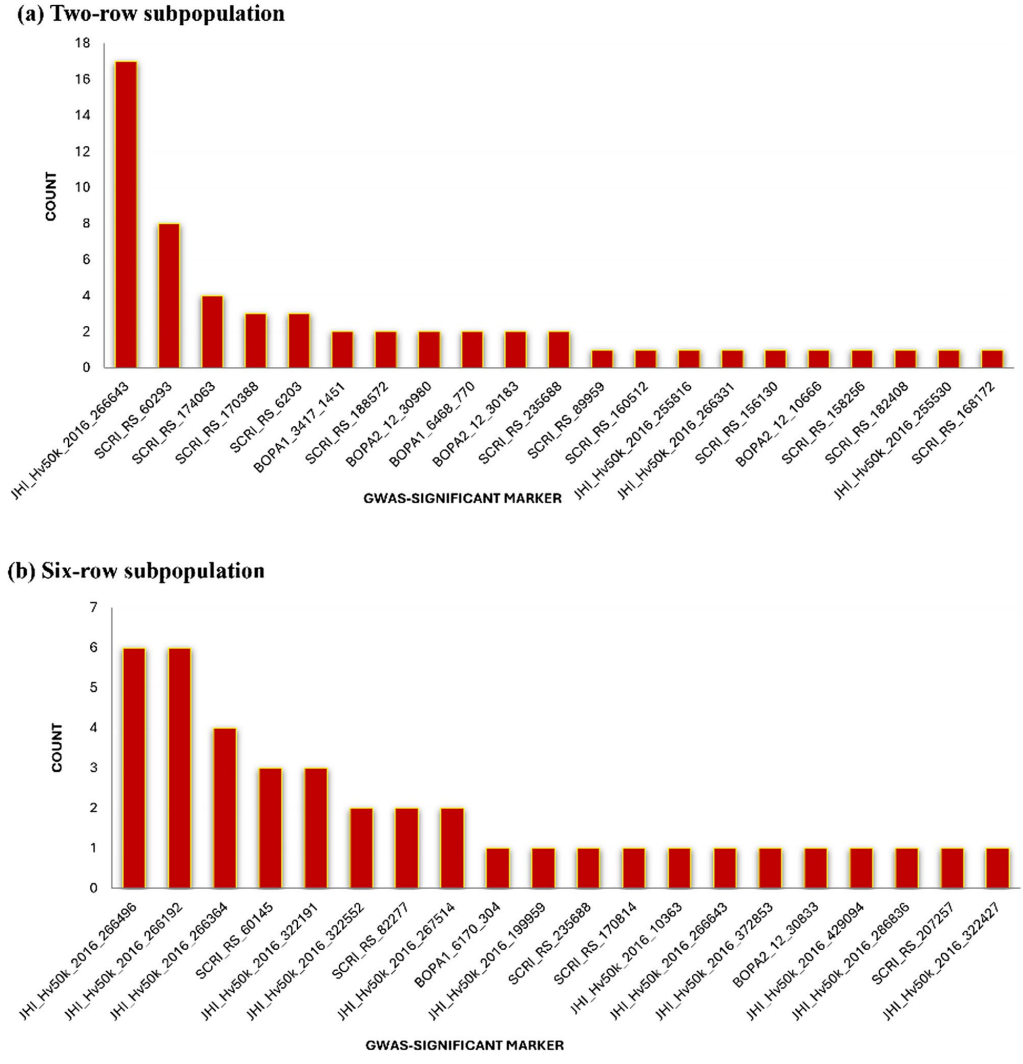


**Fig. 4** Markers selected from 20 iterations of GWAS using independent training set. **a** Number of markers detected in two-row subpopulation. **b** Number of markers detected in six-row subpopulation

In the two-row subpopulation, the average PA of STD_rrBLUP model was 0.68, with a range of 0.44 to 0.90 across 500 iterations (Fig. 5b, top right panel). For the six-row subpopulation, the model yielded an average PA of 0.71, with a broader range of 0.22 to 0.89 (Fig. 5b, bottom right panel). The GEBVs derived from both the Standard GP model and the GWAS-incorporated GP model were used to rank genotypes, forming the basis for genomic selection at predefined thresholds which were discussed below.

### Genotype selection patterns and consistency across different selection strategies

To evaluate the consistency of the genotype selection strategies for powdery mildew resistance, two selection thresholds were applied: the top 10% and 20% most resistant genotypes in each subpopulation. Thereafter, the presence of each genotype were assessed across the four selection methods: PS, wMAS, STD_rrBLUP, and GWAS_FIX_rrBLUP. The genotype selections among these four methods are here visualized using the UpSet

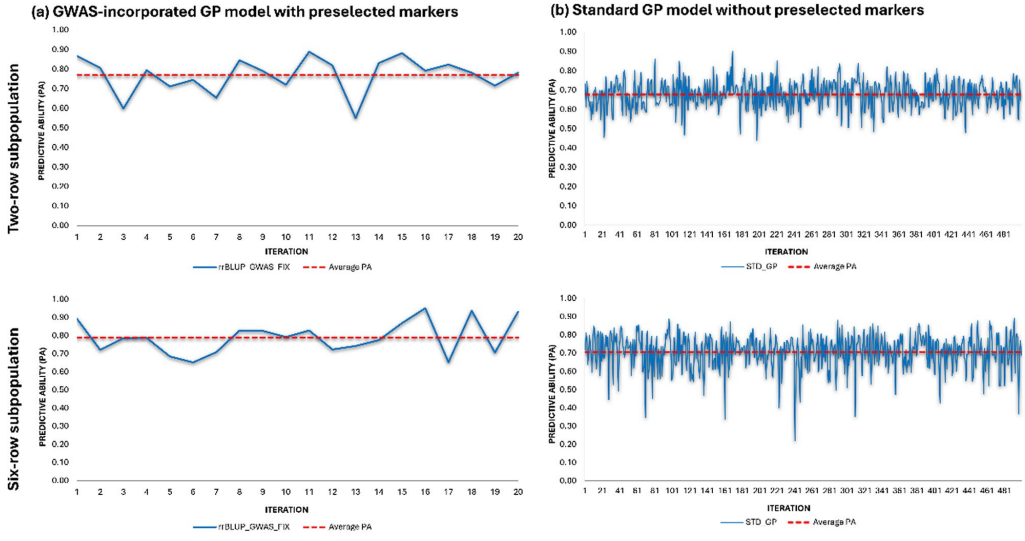


**Fig. 5** PA of two genomic prediction models in two-row (top row) and six-row (bottom row) subpopulations. The left panel (Fig. 5**a**.) represents the PA of GWAS-incorporated GP model (GWAS_FIX_rrBLUP) which included Bonferroni-significant markers as fixed effects while the remaining markers were included as random effects across 20 iterations. Right panel (Fig. 5**b**.) represents the PAs of the Standard GP model (STD_rrBLUP) across 500 iterations including all the tested markers as random effects. The yellow line represents the average PA across all iterations for each subpopulation. The average PA of two-row subpopulation were 0.68 for STD_rrBLUP and 0.77 for GWAS_FIX_rrBLUP. The average PA of six-row subpopulation were 0.71for STD_rrBLUP and 0.79 for GWAS_FIX_rrBLUP

plots (Fig. 6), while detailed information on the genotypes and their corresponding groups according to the selection methods are provided in Supplementary Tables S8 to S11.

### *Genotypes selection in two-row subpopulation*

By the use of the 10% threshold level, nine genotypes representing potential candidates for powdery mildew resistance due to both resistant phenotypes and strong genetic backgrounds were collectively chosen by all four selection methods used here (Fig. 6). A total of 139 genotypes fell outside the 10% selection threshold. Nine genotypes were selected using three selection methods, specifically through two combinations: one excluding PS and the other excluding GWAS_FIX_rrBLUP. Twenty genotypes were jointly selected by two methods (eight genotypes by PS and wMAS, nine by GWAS_FIX_rrBLUP and wMAS; three by STD_rrBLUP and wMAS). Under their own selection criteria, wMAS identified 41 unique genotypes while STD_rrBLUP identified only one genotype (Fig. 6).

Under the 20% selection threshold, 23 genotypes were commonly selected by all four selection methods. Additionally, nine genotypes were consistently identified by marker-based and genomic-based selections but were not selected by PS. Ten genotypes were co-selected by three methods where PS and wMAS were always included along with either GWAS_FIX_rrBLUP or STD_rrBLUP. Sixteen genotypes were selected by two methods, with wMAS as a primary selection approach, while the second method varied among GWAS_FIX_rrBLUP, STD_rrBLUP, and PS. Eight genotypes were uniquely selected by STD_rrBLUP, while wMAS selected 21 genotypes independently, and PS selected only one genotype. All four methods consistently excluded the same 131 genotypes (Fig. 6).

### *Genotypes selection in six-row subpopulation*

At the 10% selection threshold, 124 genotypes were not selected by any method, indicating their poor performance and limited potential for breeding. In contrast, five genotypes were selected by all methods which suggests their superior phenotypic performance and genetic background for durable resistance. Nine genotypes were selected by three methods, five genotypes by two methods in different combinations, selected five genotypes. The remaining eight genotypes were uniquely selected by only one method (Fig. 6).

At 20% threshold, the genotype selection pattern was similar to that at 10% threshold, but with more genotypes included. Nineteen genotypes were consistently selected by all methods, while the number of genotypes not selected by all methods decreased to 107. Three methods commonly selected seven genotypes, while five were

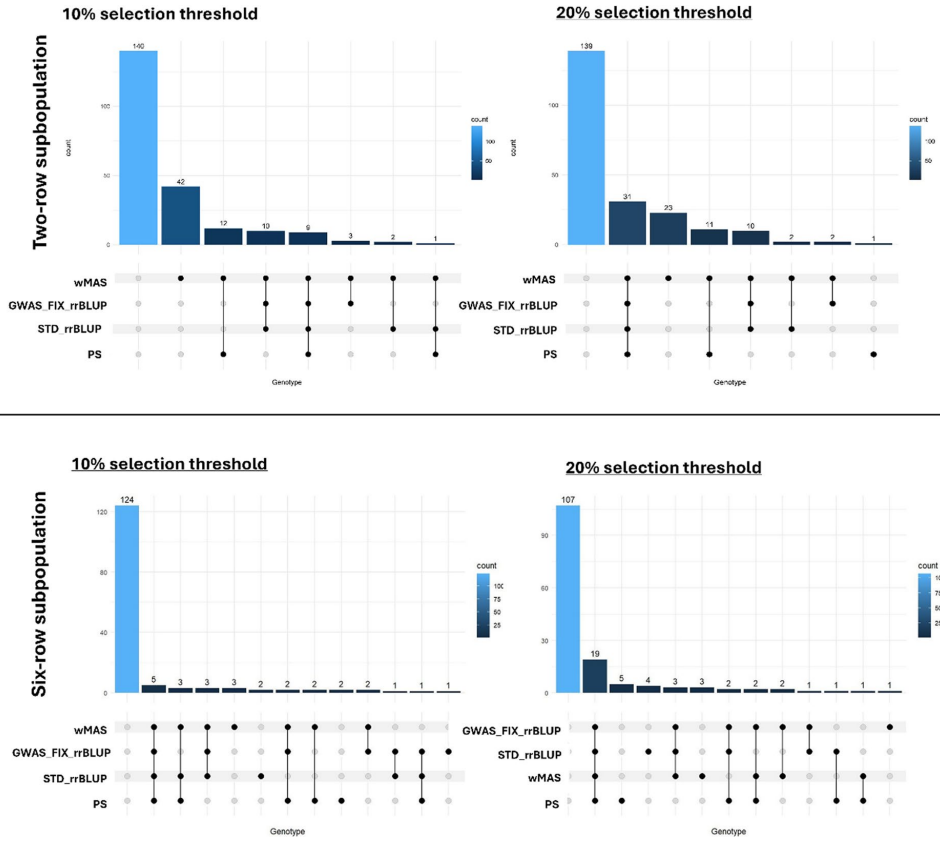


**Fig. 6** UpSet plots illustrating the genotypes selected across four selection methods (PS: phenotypic selection; wMAS: weighted marker-assisted selection; GWAS_FIX_rrBLUP: GWAS-incorporated genomic prediction; and STD_rrBLUP: standard genomic prediction) in the two-row and six-row barley subpopulations under 10% (left) and 20% (right) selection thresholds. Black circles indicate methods that selected a given genotype, whereas gray circles indicate methods that did not. Connecting lines represent genotypes selected by multiple methods, indicating shared selection options

identified by two methods, in different combinations. The remaining 13 genotypes were exclusively selected by only one method (Fig. 6).

## Discussion

The current study revealed substantial phenotypic variation for powdery mildew resistance in the evaluated spring barley population, with a high broad-sense heritability indicating genetic backgrounds as predominant factors. While most of the tested population was susceptible, the existing genetic diversity and heritable variation provide a great opportunity for effective breeding. The identification of MTAs and QTL offers a direct path to a wMAS approach. Our study found that a positive correlation between the genetic merit and BLUP values indicates wMAS effectively captured a large portion of phenotypic variation in both populations. However, relying solely on major genes has significant limitations. Due to their pleiotropic effects, negative trade-offs such as susceptibility to other pathogens and yield loss can be occurred [35, 36]. This indicates the need for broader approaches that are less dependence on major genes. To address this, genomic selection uses genome-wide markers reducing the reliance on single genes and contributing to more durable resistance [18]. Our GWAS results support this by identifying 39 markers forming 21 QTL of which several have been previously reported as summarized in Table 3. Detail information of these QTL are provided in Supplementary Table S3. A limitation to consider is that the virulence profile of the field-derived powdery mildew inoculum was not defined, meaning that part of the observed phenotypic variation may

**Table 3** Summary of identified QTL and their comparison to previously reported loci

| Identified QTL | Position (Mbp) | Population | Previously Reported QTL/Gene | Comparison to known loci | References |
|---|---|---|---|---|---|
| QTL_1H_1 | 4.18–4.94 | Both | *Mla* locus (~ 7 Mbp) & 30 described race-specific resistance specificities | Plausible a variant of *Mla* | [37–40] |
| QTL_1H_2 | 10.55 | six-row | Known QTL (11.3 Mbp) | Same QTL | [41] |
| QTL_1H_3 | 493.32 | two-row | Qrbg_1H_3 (~ 494.08 Mbp) | Same QTL | [4] |
| QTL_2H_1 | 26.54 | two-row | Known QTL (29 Mbp) | Same QTL | [41] |
| QTL_2H_2 | 41.78 | two-row | Known QTL (38.1 Mbp) | Same QTL | [41] |
| QTL_2H_3 | 263.47 | two-row | no proximal QTL | New QTL | No prior reference |
| QTL_2H_4 | 664.04 | two-row | no proximal QTL | New QTL | No prior reference |
| QTL_3H_1 | 541.7 | six-row | no proximal QTL | New QTL | No prior reference |
| QTL_4H_1 | 536.71–538.09 | two-row | no proximal QTL | New QTL | No prior reference |
| QTL_4H_2 | 570.52 | two-row | no proximal QTL | New QTL | No prior reference |
| QTL_4H_3 | 586.89–590.48 | Both | *mlo* locus (~ 589.3 Mbp) | Same QTL | [42] |
| QTL_5H_1 | 1.32–2.22 | two-row | Qhv_PM-5 H.1 (2.54 Mbp) | Same QTL | [43] |
| QTL_5H_2 | 25.89 | six-row | no proximal QTL | New QTL | No prior reference |
| QTL_5H_3 to QTL_5H_6 | ~ 500–550 | Both | *Mlj* locus (imprecise) & four different QTL | Potentially corresponds to *Mlj* | [41, 44] |
| QTL_6H_1 & QTL_6H_2 | 9.84, 37.02 | six-row | *Mlh* locus (imprecise) | Potentially corresponds to *Mlh* | [6, 44] |
| QTL_6H_3 | 554.11 | six-row | no proximal QTL | New QTL | No prior reference |
| QTL_7H_1 | 557.5 | two-row | no proximal QTL | New QTL | No prior reference |

reflect variability within the pathogen population itself. The absence of pathogen characterization could also have contributed to differences in disease progression between experiments.

Some MTAs were consistently detected across different runs, suggesting they may represent major-effect loci for further functional analysis. In contrast, MTAs identified less frequently or even once suggest they might be false positives. However, some of them may be truly associated with powdery mildew resistance, but with small effects, or they may be specific to particular genetic backgrounds. Validation using separate or larger populations would be required to confirm their usefulness in barley breeding programs.

The results showed the role of known major genes in powdery mildew resistance. Among the three QTL identified on chromosome 1 H, QTL_1H_1 is proximal to the well-known *Mla* locus suggesting it may represent a resistant variant within this major gene [37–40]. Additionally, QTL_1H_3 overlapped with a previously identified QTL, Qrbg_1H_3 and this QTL is reported to be associated with several plant defense-related proteins and enzymes [45–48]. Furthermore, two QTL detected on chromosome 2 H and most QTL on chromosome 5 H aligned with previously reported loci associated with seedling-stage resistance. The presence of the durable *mlo* gene on chromosome 4 H was also confirmed and it overlapped with QTL_4H_3 from our study. Most of the top-selected genotypes carried alleles closely linked to this region, suggesting that *mlo*-mediated resistance is present in the evaluated panel. However, since the causal *mlo* allele was not directly genotyped in this study, its presence cannot be confirmed for all individuals, and balancing *mlo*-based powdery mildew resistance with potential agronomic trade-offs remains important. While the precise position of the known *Mlh* locus is not available, though it is generally found near the short arm of chromosome 6 H. QTL_6H_1 and QTL_6H_2 from the current study may correspond to it since they were also proximal to this region [6, 44]. In addition to validating known loci, our study identified eight new QTL associated with powdery mildew resistance. These newly detected loci were located on chromosomes 2 H, 3 H, 4 H, 5 H, 6 H, and 7 H, consistent with the positions detailed in Table 3. Notably, some QTL such as QTL_3H_1 and QTL_7H_1, colocalized with QTL previously reported as adult-plant resistance. This suggests that these QTL are valuable for broadening resistance spectrum throughout the plant's life cycle. Validation of these novel QTL will require testing in biparental or multi-parent populations, which was beyond the scope of the current study; however, such evaluation remains important for future validation efforts.

Previous studies have shown different results, some reported that including specific markers as fixed effects in genomic prediction models improved prediction accuracy while others found no effect or reduced accuracy [13, 16, 49–52]. In the present study, performance ranking among the genomic selection methods resulted in that the GWAS-incorporated GP model consistently achieved the highest PA, followed by wMAS, with the Standard GP model having the lowest PA. The superior

performance of the GWAS-incorporated GP model is due to its effective use of GWAS-significant markers and that it optimizes the prediction [50]. The better outperformance of wMAS compared to the Standard GP model reflects the dominance of major-effect markers on powdery mildew resistance in these subpopulations. In the Standard GP model, all markers are uniformly shrunk toward zero, which reduces the effects of major loci and weakens predictions when dominant genes are present [34, 50].

The frequently detected MTAs observed in both subpopulations highlights the presence of a consistent genetic association with powdery mildew resistance in the full set of genotypes. However, MTAs detected only in random subsets of genotypes rather than the full subpopulation are likely population-specific and can be overlooked in broader analyses. Apart from these marker results, the different genetic architecture between the two subpopulations also explains the overall performance of the genomic prediction models. The six-row subpopulation outperformed the two-row subpopulation in both genomic prediction models, and this is likely due to the presence of both major genes and a favorable polygenic background. The ability of genomic prediction models to capture the cumulative effect of small-effect loci and the major genes, contributes to a high overall predictive accuracy [10, 52]. Additionally, wMAS was also notably effective in both subpopulations, showing a high correlation between genetic merit and BLUP values. However, genomic prediction appears more suitable as it has a more complex genetic architecture captured by genome-wide prediction models whereas resistance in the two-row subpopulation is mainly controlled by major-effect markers.

In addition to the different genetic architecture of the two subpopulations, the genotype selection patterns also indicated a fundamental difference. In the two-row subpopulation, a large group of genotypes were exclusively selected by wMAS revealing the dominance of major-effect QTL. This exclusive selection highlights that these genotypes have an unfavorable polygenic background and therefore they were not selected by the other methods [53]. In contrast, the six-row subpopulation exhibited a more diverse genetic architecture. The wMAS-exclusive group was smaller compared to the two-row subpopulation which suggests a strong polygenic background in the six-row subpopulation. These architectural differences were further reflected in how genotypes were selected under different thresholds.

When the selection threshold was relaxed, both the number of selected genotypes and the overlap among methods increased. Additionally, it allowed borderline genotypes with different combinations of major and minor effects, or strong phenotypic performance, to be included by multiple selection methods. Analysis of selection patterns between phenotypic and genetic approaches showed that genetic analyses can detect hidden signals that may be masked or only partly expressed during phenotypic evaluation. The differences between phenotypic and genomic selections may be due to environmental variation. This includes factors like temperature, light distribution, moisture, and humidity [54, 55].

The contrasts were not only driven by environmental variation but also by the different assumptions and algorithms underlying each selection method. For example, the GWAS-incorporated GP model can reduce its flexibility by explicitly fixing major-effect markers without shrinkage. As a result, the model may overfit and fail to predict genotypes that depend more on polygenic backgrounds [52, 56, 57]. In addition, some genotypes were consistently identified by phenotypic selection, wMAS, and the GWAS-incorporated GP, but not by the Standard GP. This shows how the Standard GP model underestimates genotypes with major-effect alleles since it shrinks all marker effects toward zero [34, 50].

These differences in selection outcomes are important when considering breeding strategy. In the two-row subpopulation, wMAS could be useful to rapidly select genotypes that carry the major-effect QTL. However, to develop more robust and durable resistance, a whole-genome approach is necessary to select genotypes with a superior polygenic background. In the six-row subpopulation, resistance comes from diverse resistance mechanism and therefore genomic selection is a more suitable option for long-term improvement than relying on a single dominant locus.

## Conclusions

This study suggests that incorporating GWAS-identified markers into genomic prediction models can improve prediction accuracy for powdery mildew resistance in spring barley. The identified QTL from this study can be validated across multiple locations and also can be incorporated into breeding pipelines to develop powdery mildew resistant cultivars. Since these QTL were detected at the seedling stage, validating their effects at the adult-plant stage could be done as future work. Furthermore, developing multi-trait genomic prediction models that include powdery mildew resistance together with traits such as yield could provide breeders with a more efficient selection strategy.

**Abbreviations**

| | |
|---|---|
| QTL | Quantitative Trait Loci |
| AUDPC | area under disease progress curve |
| MTAs | marker-trait associations |
| GWAS | genome-wide association study |
| GP | genomic prediction |
| PA | predictive ability |
| GEBV | genomic estimated breeding values |

| | |
|---|---|
| PS | phenotypic selection |
| wMAS | weighted marker-assisted selection |
| GS | genomic selection |
| DPI | days post inoculation |
| FDR | False discovery rate |
| MAF | minor allele frequency |
| LD | linkage disequilibrium |
| BLUP | best linear unbiased prediction |
| GWAS_FIX_rrBLUP | GWAS-incorporated GP |
| STD_rrBLUP | Standard GP |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-12395-y.

Supplementary Material 1.

Supplementary Material 2.

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Not applicable.

**Authors' contributions**
Su Myat Noe, Firuz Odilbekov, and Aakash Chawade conceived of the study. Aakash Chawade received the funding. Su Myat Noe conducted greenhouse experiments and evaluated powdery mildew disease at seedling stage. Firuz Odilbekov provided genotypic data for the study. Su Myat Noe analyzed data and wrote the first draft. Su Myat Noe, Firuz Odilbekov, Aakash Chawade, Pawan Singh, and Eva Johansson reviewed the manuscript. All authors read and approved the final version of this manuscript.

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**Data availability**
The phenotypic data sets, GWAS results and genomic prediction results that support the findings of this study are provided in additional files. All significant SNPs associated with powdery mildew resistance are included in the Supplementary Materials (Supplementary Table S3). The genotypic datasets analyzed during the current study are not publicly available due to intellectual-property restrictions owned by Lantmännen, our industry partner, but are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
This article does not contain any studies with human participants or animals. The collection materials of the plants comply with the relevant institutional, national, and international guidelines and legislation.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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## Publisher's note

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This thesis investigated the genetic basis of three foliar diseases in spring barley using GWAS and genomic prediction across diverse panels. Multiple resistant loci were identified, including novel ones. The integration of GWAS-derived markers into genomic prediction models enhanced prediction accuracy for powdery mildew resistance, with major-effect loci contributing significantly. Additionally, prediction performance for net form net blotch resistance was influenced by factors such as linkage disequilibrium patterns, trait heritability, and the genetic compatibility between training and validation populations. These findings highlight the importance of population composition and analytical strategies in enhancing disease resistance breeding.

**Su Myat Noe** received her doctoral education at the Department of Plant Breeding, Swedish University of Agricultural Sciences (SLU), Alnarp. She holds a master's degree in Plant Biology from the Swedish University of Agricultural Sciences (SLU), Uppsala. She also holds a master's degree and a bachelor's degree in Agricultural Science from Yezin Agricultural University, Myanmar.

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