

A novel barley leaf rust resistance locus, distinct from Rph5 and Rph7, identified on barley chromosome 3HS

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Abstract

Barley leaf rust (BLR), caused by *Puccinia hordei*, is a major foliar disease that threatens barley production worldwide, causing substantial yield and quality losses. The identification, genetic characterization and utilisation of novel resistance genes are critical to diversification, achieving prolonged resistance and reducing dependence on fungicides. In this study, we discovered and characterized All Stage Resistance (ASR) to *P. hordei* in six international barley genotypes from the Middle East. Genetic analyses revealed digenic inheritance in four genotypes (AGG-87, AGG-102, AGG-402, and AGG-514) and monogenic inheritance in two genotypes (AGG-72 and AGG-137). Based on selective genotyping analysis, resistance in AGG-137 (temporarily designated *RphAGG-137*) was mapped to chromosome 3HS spanning 3.62 to 9.83 Mb on the MorexV3 reference genome assembly. Extended genetic analysis, multi-pathotype tests, marker genotyping and mapping demonstrated that AGG-137 in fact carries *Rph2* plus *RphAGG-137*, the latter being distinct from *Rph5* and *Rph7* reported on 3HS. The resistance in AGG-72 (*RphAGG-72*) was mapped to chromosome 4H, encompassing the genomic region 554.68 to 577.32 Mb in the same region as *Rph21* and *RphCas* (a provisionally designated ASR gene in the eastern European barley cultivar Caspian) implying that *RphAGG-72* is either one of these two genes or new.

Introduction

Barley leaf rust (BLR), caused by the fungus *Puccinia hordei* (*Ph*) is one of the most prevalent and economically significant diseases affecting barley (*Hordeum vulgare*) globally. The disease can cause yield losses ranging from 10–40% but under favourable conditions, reductions of up to 60% have been reported (Park et al., 2015; Singh et al., 2025). Leaf rust epidemics diminish photosynthetic capacity by causing foliar damage, leading to shrivelled grains and reduced grain quality (Liu et al., 2025). The deployment of resistant cultivars is the most sustainable and cost-effective management strategy; nevertheless, the emergence of new *Ph* pathotypes that overcome existing resistance genes poses a persistent challenge for breeders. Consequently, identifying and mapping novel sources of resistance is imperative for diversifying genetic base of resistance and reduced reliance on chemical control.

Identification of potentially new resistance to rust in cereals typically involves preliminary multipathotype testing, followed by genetic analyses based on biparental mapping populations segregating for the resistance. Phenotyping with relevant rust isolates and genotyping is then conducted, followed by comprehensive trait association analysis to pinpoint the targeted resistance locus. The type and density of molecular markers used for mapping determines the resolution of the maps generated and phenotypic accuracy often affects the accuracy of mapping new resistance genes. In the last decade, several BLR resistance genes have been mapped/fine-mapped in bi-parental mapping populations using different genotyping platforms and marker systems to determine the genomic location of interest, e.g., *Rph13* (medium resolution fine mapping using SNPs - Jost et al., 2020), *Rph23* (QTL mapping using SSRs - Singh et al., 2015), *Rph27* (bulk segregant analysis using DArT - Rothwell et al., 2020), *Rph28* (CAPs - Mehnaz et al., 2021a). Attempts to identify new sources of resistance without a detailed understanding

of pathogen virulence and the genetic basis of resistance frequently results in selecting the wrong genes in breeding populations. Determining the initial chromosomal location facilitates subsequent fine mapping and cloning studies and results in a more comprehensive understanding of the structural and functional characteristics of resistance genes. Molecular markers in linkage disequilibrium with the gene derived from gene mapping can be effectively utilised in marker-assisted selection (MAS) to enhance plant breeding efficiency, while gene pyramiding can be employed to achieve long-lasting resistance.

Previous studies by Mehnaz et al., (2021b) identified several novel sources of all stage resistance (ASR) from a large barley collection assembled from the Middle East. Among these sources, nine genotypes were found to carry broad spectrum resistance that was effective to all prevalent Australian *Ph* pathotypes. Resistance in three of these genotypes (AGG-396, 397, 398) was mapped to chromosome 2H and found to be either new or allelic to resistance gene *Rph14* (Mehnaz et al., 2022). The present study aimed to characterize resistance in the remaining six genotypes (AGG-72, AGG-87, AGG-102, AGG-137, AGG-402, AGG-514). In the first objective of the study, we established six biparental mapping populations (derived from Resistant (R)/Susceptible (S) crosses) and conducted inheritance studies. The second objective of the study aimed at mapping resistance and identifying the genomic regions associated with it in two genotypes (AGG-72 and AGG-137) that were found to exhibit monogenic inheritance.

Materials and methods

Plant and pathogen material

The six test lines (AGG-72, AGG-87, AGG-102, AGG-137, AGG-402, AGG-514) were sourced from the Australian Grains Gene (AGG) Bank and maintained in the seed storage facility unit of the Plant Breeding Institute Cobbitty (PBIC), University of Sydney. Passport details and the ID of each line as maintained by AGG are provided in Supplementary Table 1. Barley genotype Gus was used as a leaf rust susceptible control or for crossing to establish biparental mapping populations. Twelve pathotypes of *Ph* with contrasting virulence/avirulence spectra were used in this study, depending on the requirements of the experiments (Table 1). All pathotypes originated from annual pathogenicity surveys of *Ph* conducted in Australia and are preserved in liquid nitrogen at PBIC. The virulence and avirulence profiles of the pathotypes are documented in Table 1.

Table 1
Puccinia hordei pathotypes used in this study including culture number and virulence profile

Pathotypes*	PBI culture	Virulence profile
200 P-	518	<i>Rph8, Rph25</i>
220 P+	485	<i>Rph5, Rph8, Rph19, Rph25</i>
220 P + + <i>Rph13</i>	577	<i>Rph5, Rph8, Rph19, Rph13</i>
222P+	545	<i>Rph2, Rph5, Rph8, Rph25</i>
253 P-	490	<i>Rph1, Rph2, Rph4, Rph6, Rph8, Rph25</i>
5652 P+	561	<i>Rph2, Rph4, Rph6, Rph8, Rph9, Rph10, Rph12, Rph19, Rph25</i>
5610 P+	637	<i>Rph4, Rph8, Rph9, Rph10, Rph12, Rph19, Rph25</i>
5672 P+	639	<i>Rph2, Rph4, Rph5, Rph6, Rph8, Rph9, Rph10, Rph12, Rph19, Rph25</i>
5453 P-	560	<i>Rph1, Rph2, Rph4, Rph6, Rph9, Rph10, Rph12, Rph25</i>
5457 P-	626	<i>Rph1, Rph2, Rph3, Rph4, Rph6, Rph9, Rph10, Rph12, Rph19, Rph25</i>
5457 P+	612	<i>Rph1, Rph2, Rph3, Rph4, Rph6, Rph9, Rph10, Rph12, Rph25</i>
5477 P-	672	<i>Rph1, Rph2, Rph3, Rph4, Rph5, Rph6, Rph9, Rph10, Rph12, Rph25</i>
*Pathotype designation is based on the virulence or avirulence pattern of a pathotype on a differential set using the octal notation system proposed by Gilmour (1973). The symbols P- and P+ indicate avirulence and virulence, respectively, on the barley cultivar Prior carrying <i>Rph19</i> (Park 2003). <i>Rph25</i> virulence is based on virulence in cultivar Fong Tien carrying <i>Rph25</i> .		

Development of mapping populations

Six crosses (AGG-72/Gus, AGG-87/Gus, AGG-102/Gus, AGG-137/Gus, AGG-402/Gus and AGG-514/Gus) were advanced to the F₂ generation. The F₂ single plants were space planted in the field and harvested as F₃ families [AGG-72/Gus (n = 113), AGG-87/Gus (n = 117), AGG-102/Gus (n = 105), AGG-137/Gus (n = 116), AGG-402/Gus (n = 98), AGG-514/Gus (n = 81)].

Sowing and inoculations

Fifteen to 20 seeds from each F₃ family along with parents and a BLR differential set were sown as clumps in 90mm diameter pots (3 lines/pot) containing a potting mix of fine pine bark and coarse sand. Prior to sowing, all pots were fertilized with Aquasol® (@25g/10L of water). After sowing, pots were kept

at 24°C in a temperature-controlled growth room. Ten-day old seedlings were inoculated with relevant *Ph* pathotypes listed in Table 1. A suspension mixture was prepared by adding 10 mg urediniospores /10 ml of isopar- a light mineral oil (Univar®, Ingleburn, NSW, Australia) for 200 pots and the mixture was then atomised over the seedlings with a mist atomizer. Following inoculation, seedlings were incubated at an ambient temperature (ranging from 16-22°C) in a dark chamber for 24 hours. An ultrasonic humidifier was used to create mist in the incubation chamber. After 24 hours of incubation, seedlings were shifted to microclimate rooms maintained at 24 ± 2°C with natural light and an automated irrigation system.

Disease scoring and genetic analysis

Between 15 to 20 seeds from individual F₃ families derived from each of the populations were assessed in the greenhouse for response to pathotype 5457 P+ (current predominant pathotype in Australia with broad spectrum virulence for genes *Rph1*, *Rph2*, *Rph3*, *Rph4*, *Rph6*, *Rph9*, *Rph10*, *Rph12*, *Rph19* and *Rph25*). Plants were scored when the susceptible control Gus reached infection type (IT) of “3+” (fully compatible or susceptible) using the Stakman “0”-“4” IT scale. F₃ families were scored as non-segregating resistant (NSR), non-segregating susceptible (NSS), or segregating (Seg) for resistance and susceptibility. The data obtained from the greenhouse rust screening was subjected to Chi-squared analysis (χ^2) at a significance level of $\alpha = 0.05$ to ascertain the goodness of fit of the observed ratios with the expected genetic ratios.

DNA extraction and genotyping

DNA extraction and genotyping

Genomic DNA was extracted from individual (single plant) leaf tissues of F₃ families of AGG-72/Gus and AGG-137/Gus populations including resistant and susceptible parents using a CTAB protocol (Fulton et al., 1995). The concentration of DNA was determined using a spectrophotometer (Nanodrop™, Biolab, Melbourne, VIC, Australia). The quality of the DNA was assessed by running all samples on a 0.8% agarose gel. DNA samples from individual plants belonging to the NSR and NSS families of AGG-72/Gus and AGG-137/Gus were diluted to 100 ng/ul and subsequently dispatched to Agriculture Victoria Research, Bundoora, Australia for genotyping.

Selective genotyping and candidate region identification

Selective genotyping was performed using resistant and susceptible progeny groups from the AGG-72/Gus and AGG-137/Gus populations classified following phenotyping with pathotype 5457 P+. The AGG-72/Gus analysis used 16 NSR and 16 NSS F₃ progeny and AGG-137/Gus analysis used 26 NSR and 28 NSS F₃ progeny. Genotyping was performed using the barley targeted genotyping-by-sequencing assay (tGBS; HvGBSv2.1 ; Ge et al., 2021) for the AGG-137/Gus population, and the Infinium™ Wheat Barley 40K v1 SNP array (assay Keeble-Gagnère et al., 2021) for the AGG-72/Gus population.

Putative linkage was assigned for markers having contrasting consensus genotypes calls across the resistant and susceptible progeny groups, call rates of at least 60% in both progeny groups, a call rate difference up to 30% between groups, and at least 80% fixation of genotype calls within each of the resistant and susceptible groups. Genomic regions of interest were prioritised when at least two putatively trait-linked markers occurred within 20 Mb of one another, based on the Morex v1.0 genome assembly (Mascher et al., 2017).

Marker frequency analysis of the AGG-137/Gus population

Marker frequency analysis (MFA) was performed using 16 NSR and 7 NSS F3 progeny from the AGG-137/Gus population classified following phenotyping with pathotype 220 P + + *Rph13* and for which tGBS genotype data were available. Marker-trait association was assessed by comparing allele frequencies between the resistant and susceptible phenotypic classes. A discriminant value was calculated to quantify allelic differentiation between the two classes as described by Wenzl et al., (2007).

Positioning reported markers and published *Rph* loci on MorexV3

Original physical positions of the reported markers were based on the Morex v1.0 reference assembly. To update these positions to MorexV3 (Mascher et al., 2021), marker flanking sequences were queried against the MorexV3 pseudomolecules using BLASTN via the GrainGenes SequenceServer (Priyam et al., 2019). Searches used an E-value threshold of 1×10^{-5} , match score = 2, mismatch penalty = - 3, gap-open penalty = 5, gap-extension penalty = 2, and low-complexity filtering. The polymorphic site in each query was represented using the corresponding IUPAC ambiguity code. For each marker, the best unambiguous hit on the expected chromosome was retained, and the MorexV3 coordinate of the polymorphic site was recorded, accounting for reverse-strand alignments where required. Only the reported markers were projected; the complete marker dataset was not realigned or reanalysed. Original Morex v1.0 coordinates for the markers associated with the AGG-137/Gus and AGG-72/Gus candidate regions are retained in Supplementary Table 2 for traceability. Published positions of designated *Rph* loci occurring within or overlapping the candidate regions of interest were obtained from Clare et al., (2024), which consolidated barley rust resistance loci against the MorexV3 reference genome.

Marker genotyping

Based on greenhouse response to eight *Ph* pathotypes (Table 2), it was hypothesized that the test lines carry either uncharacterized seedling resistance (USR) or known resistance that could not be postulated with the array of pathotypes used. At the time when this study was conducted, virulence for genes *Rph7*, *Rph14*, *Rph15* and *Rph21* had not been detected in Australia (Park et al., 2015), virulence for *Rph7* was subsequently detected in 2020 (Park RF unpublished). To rule out the possible presence of *Rph7* and *Rph15*, the six lines were genotyped with a KASP marker diagnostic for *Rph7* (Chen et al., 2023) and *Rph15* (Chen et al., 2021). Because predictive molecular markers linked to *Rph14* (Golegaonkar et al.,

2009) and *Rph21* (Sandhu et al., 2012) are not available, marker assays could not be performed for the presence of these two genes.

Table 2

Seedling infection type responses conditioned by six resistant genotypes and the leaf rust susceptible barley genotype Gus in response to eight *P. hordei* pathotypes

Genotype	Pathotypes							
	200 P-	220 P+	253 P-	5652 P+	5610 P+	5453 P-	5457 P- P- P-	5457 P + P+
AGG-72	;12	;12	;CN	;1-	;	;12	;C	;C
AGG-87	;12	;12 ;1c	;1C	;12	;12	;12	;1 + C	;1C
AGG-102	;CN	;1C	;1C	;12C	;1C	;1C	;1C	;1CN
AGG-137	;	;1 = C	;+C	;+C	;+C	;+C	;+C	;+C
AGG-402	;1	;1C	;1CN	;N	2C	2CN	2C	;1CN
AGG-514	;1+	;1+	;+N	;1-CN	;CN	;CN	;CN	;CN
Gus (Sus)	3+	3+	3+	3+	3+	3+	3+	3+
Infection types are categorised on a '0' - '4' scale (Park and Karakousis, 2002). Hypersensitive flecks are represented by the symbol ';', small uredinia enclosed by necrotic tissues by '1', medium-sized uredinia enclosed by chlorotic and/or necrotic tissues by '2', large uredinia with or without chlorosis by '3', and susceptible hosts by '3+'. Necrosis is represented by 'N', chlorosis by 'C', and symbols '+' and '-' indicate higher and lower infection types than normal, respectively. Gus was used as a susceptible check.								

Results

Phenotyping and marker genotyping of the parents

All six resistant parents included in this study produced very low to intermediate ITs ("0;" to "2") when tested against eight pathotypes (Table 2). In contrast, the susceptible parent, Gus, produced high IT of "3+" to all eight pathotypes. All parents in this study tested negative for markers linked to *Rph7* and *Rph15*, indicating that resistance in these lines is not conferred by *Rph7* and/or *Rph15*.

Genetic analyses of mapping populations

Phenotypic data obtained through greenhouse testing of the F₃ families with pathotype 5457 P+ indicated monogenic segregation patterns (1 NSR : 2 Seg :1 NSS) for two populations (AGG-72/Gus (P > 0.8) and AGG-137/Gus (P > 0.9)), and digenic inheritance (7 NSR : 8 Seg : 1 NSS) for four populations (AGG-87/Gus, AGG-102/Gus, AGG-402/Gus and AGG-514/Gus) (Table 3).

Table 3
Distribution and Chi-squared analysis of F₃ families derived from six crosses when rust tested against *P. hordei* pathotype 5457 P + as seedling in greenhouse

Population	NSR*	Seg	NSS	Genetic ratio	χ^2	P
AGG-72/Gus	31	54	28	1:2:1	0.38	0.82
AGG-87/Gus	52	59	6	7:8:1	0.25	0.88
AGG-102/Gus	49	53	3	7:8:1	2.14	0.34
AGG-137/Gus	29	60	27	1:2:1	0.21	0.90
AGG-402/Gus	33	60	5	7:8:1	4.90	0.08
AGG-514/Gus	44	33	4	7:8:1	3.68	0.16
*NSR = non segregating resistant; Seg = Segregating; NSS = non segregating susceptible						
χ^2 value for significance at $P = 0.05$ is 5.99 at 2 d.f.						

Mapping of leaf rust resistance in AGG-137 and AGG-72

Selective genotyping located resistance in AGG-137 (tentatively designated *RphAGG-137*) on the short arm of chromosome 3H between 3.62 and 9.83 Mb MorexV3 (Table 4; Fig. 1). Within this region, 11 putatively trait-linked markers were identified with a fixation range 0.88 to 1.00 (Supplementary Table 2). Four genes conferring resistance to *Ph* have been reported on chromosome 3H, of which *Rph5*, *Rph6* and *Rph7* are located on 3HS in the same genomic region as *RphAGG-137* while the fourth gene *Rph10* is reported on 3HL. The pathotype 5457 P+ used for screening of AGG-137/Gus is virulent on *Rph6* and *Rph10* and avirulent with respect to genes *Rph5* and *Rph7*. Screening of the resistant parent AGG-137 with a marker closely linked to *Rph7* was negative, excluding the presence of *Rph7* in this line.

Table 4

Candidate regions identified in the AGG-137/Gus and AGG-72/Gus populations. Regions are reported as the span of high confidence markers on the MorexV3 physical map. Number of high confidence markers, call rate and fixation ranges are provided for each region. Full marker details are presented in Supplementary Table 2.

Candidate region	Population	Chr	Region (Mb)	No. mkr	Call rate range	Fixation range
<i>RphAGG-72</i>	AGG-72/Gus	4H	554.68-577.32	3	0.64-1.00	0.80–0.89
<i>RphAGG-137</i>	AGG-137/Gus	3H	3.62–9.83	11	0.61–0.77	0.88-1.00

To check the possible presence of *Rph5*, the parent AGG-137 and its derived population was tested with pathotype 220 P + + *Rph13* (virulent on *Rph5* and *Rph13*). The segregation ratios demonstrated a monogenic inheritance with this pathotype. Because pathotype 220 P + + *Rph13* is virulent on *Rph5*, the

single gene segregation observed with this pathotype suggested that the resistance in AGG-137 is distinct from *Rph5*.

The resistance in AGG-72 (tentatively designated *RphAGG-72*) was located on chromosome 4H in the genomic region spanning 554.68–577.32 Mb on the MorexV3 reference assembly (Table 4; Fig. 2). Within this region, three markers were identified with a fixation range 0.80 to 0.89 (Supplementary Table 2). *Rph21* has been reported in the same genomic region (Sandhu et al., 2012), suggesting that AGG-72 could possibly be *Rph21*.

Marker frequency analysis (MFA) in the population AGG-137/Gus

Only those NSR and NSS lines for which tGBS were available were included in MFA. Therefore, phenotypic data from only 16 NSR and 7 NSS lines were compared with genotypic data, reducing confidence in the robustness of the marker trait association analysis. Based on this analysis, two markers, chr5H|HvGBSv2-3796_125036759 + 96 and chr5H|HvGBSv2-3796_125036759 + 149, showed a discriminant value of 1.00. The markers were located at 125.036855 and 125.036908 Mb, respectively, on Morex v1.0, corresponding to 110.538275 and 110.538328 Mb on MorexV3 on chromosome 5HS.

Genes *Rph2* and *Rph25* have been reported on chromosome 5HS and 5HL respectively and are effective to pathotype 220 P+ *Rph13*+. To determine whether the locus involved might be *Rph2*, the parental line and population were tested with pathotype 5477 P- (virulent on *Rph2*, *Rph5* and *Rph25*). The parent and all lines were susceptible to pathotype 5477 P- (Fig. 3), indicating that resistance in AGG-137 against pathotype 220 P+ *Rph13* + mapped to chromosome 5HS is likely due to *Rph2*, and that pathotype 5477 P- is also virulent on *RphAGG-137*. When the parental line AGG-137 was tested with 222 P+ (also virulent on *Rph2*, *Rph5* and *Rph25*), it was resistant and the population segregated for a single gene (*RphAGG-137*). This demonstrated that AGG-137 carries *Rph2* plus an additional gene (*RphAGG-137*) on chromosome 3HS that differs from *Rph5*. The results also imply that four of the five *Rph2*-virulent pathotypes used in this study (222 P+, 5652 P+, 5453 P-, 5457 P-, 5457 P+) are avirulent on *RphAGG-137*, and that pt. 5477 P- is virulent for this gene. In contrast, pathotype 220 P+ + *Rph13* is avirulent on *Rph2* and virulent on *RphAGG-137* based on single gene inheritance (*Rph2*) observed with this pathotype.

Discussion

Understanding the inheritance of resistance and localising genomic loci/regions governing resistance are fundamental to the efficient deployment of diverse sources of resistance to control leaf rust in barley. The precision of such work is entirely dependent on the availability and use of pathotypes with accurately defined virulence. In this study, we identified and characterized resistance to *Ph* in six international barley genotypes from the Middle East, a diverse region of the fertile crescent considered to carry high genetic diversity and an important reservoir of novel alleles for resistance to *Ph* (Mehnaz et al., 2022). Genetic analyses of six genotypes based on phenotypic response to pathotype 5457 P+, virulent

on most known *Rph* genes, revealed digenic inheritance in four genotypes (AGG-87, AGG-102, AGG-402, and AGG-514) and monogenic inheritance in two genotypes (AGG-72 and AGG-137). Based on race-specificity and mapping, the leaf rust resistance in AGG-137 appeared novel and uncommon.

AGG-137 is a breeding line (pedigree unknown; listed as a MMG1273.10) introduced to Australia from Syria, that conditions strong ASR (ITs “0;” to “;1 = C”) to all Australian pathotypes against which it has been tested. Initially, we hypothesised that it harbours a single gene, provisionally designated as *RphAGG-137*. Based on selective genotyping analysis, *RphAGG-137* was mapped to chromosome 3HS spanning 3.62 to 9.83 Mb on the MorexV3 reference genome assembly (Mascher et al., 2021). Previously mapped seedling *Rph* genes on chromosome 3H include *Rph5*, *Rph6* and *Rph7* (Mammadov et al., 2003; Zhong et al., 2003; Brunner et al., 2000). *Rph7* was considered to be on the same locus as *Rph5* (Roane and Starling 1970) and *Rph5* is reported to be allelic to *Rph6* (Zhong et al., 2003). Martin et al., (2020) located *Rph5/Rph6* and *Rph7* to the terminal region of the short arm of chromosome 3H within a physical interval of approximately 1 to 10 Mb based on the Morex reference v1.0. The original Morex v1.0 coordinates of the AGG-137 candidate region span approximately 3.83–12.35 Mb (Supplementary Table 2), overlapping the ~ 1–10 Mb *Rph5/Rph6/Rph7* region reported by Martin et al., (2020). Chen et al., (2023) isolated and cloned *Rph7* reporting it as a pathogen-induced, non-canonical resistance gene and developed highly diagnostic *Rph7*-linked KASP markers. Screening of AGG-137 with these *Rph7* linked KASP markers indicated that the underlying resistance in this genotype is unlikely to be *Rph7*. At the time this study was initiated, virulence for *Rph7* had not been detected in Australia. *Rph7* virulence was reported for the first time in 2020 in a pathotype 5553 P+ (Singh et al., 2025). When AGG-137 was tested with this pathotype, the resistance in it remained unchanged further supporting marker analysis that resistance in AGG-137 is distinct from *Rph7*.

The response of AGG-137 to pathotype 5457 P+ (avirulent on *Rph5*) and mapping of this resistance to the overlap of the AGG-137 interval detected on the Morex v 1.0 reference assembly via tGBS genotyping with the *Rph5* region reported by Martin et al., (2020) suggested that AGG-137 may carry *Rph5*. When tested with pathotype 220P + + *Rph13*, a slightly altered low IT response (IT “;1 = C”) on line AGG-137 was noted. Further testing of AGG-137/Gus F₃s with pathotypes 220P+ *Rph13* + and 222 P+ (both virulent on *Rph5*) demonstrated a single gene segregation ratio to both. This suggests that resistance in AGG-137 to these pathotypes is governed by a gene that is distinct from *Rph5*. Contrasting virulence for *Rph2* between these two pathotypes also indicated that the resistance of AGG-137 is distinct from *Rph2*. The results obtained imply that pathotype 220 P + is virulent on *RphAGG-137*, and all *Rph2*-virulent pathotypes used in the study are avirulent on *RphAGG-137* except 5477 P-. It is possible that the locus detected with pathotype 5457 P + is *Rph5* and the locus detected with 222 P + is an allele of *Rph5* affective against this pathotype.

To test if the second gene in AGG-137 is *Rph2*, a MFA was conducted based on NSR and NSS F₃ families. The resistance locus was thus mapped to chromosome 5HS at approximately 110.54 Mb on MorexV3 (corresponding to 125.04 Mb on Morex v1.0). *Rph2* (Borovkova et al., 1997) maps to chromosome 5HS in the same region (Rothwell et al., 2020), indicating that the second gene in AGG-137 is likely *Rph2*,

consistent with the postulation of this gene based on pathotype analysis. *Rph2* was projected by Clare et al., (2024) to a broad interval spanning 34.08–312.74 Mb on chromosome 5H in MorexV3, encompassing the two MFA markers at approximately 110.54 Mb. Accurate marker trait associations detected by MFA should include at least 40 representatives of the phenotypic classes resistant and susceptible (Wenzl et al., 2007). Because GBS data were not available for all NSR and NSS families that were phenotyped with pathotype 220 P+ *Rph13*+, we were only able to compare 16 NSR and 7 NSS families. Consequently, only two markers with a discriminant value of 1.00 were detected on chromosome 5HS. The gene *Rph25* (Kavanagh et al., 2017) and alleles *Rph9/9.am/Rph12* (Dracatos et al., 2014) are also located on chromosome 5H but on the long arm excluding out the presence of these loci in AGG-137.

The resistance locus *RphAGG-72* in AGG-72 was mapped to chromosome 4H, encompassing the genomic region 554.68 to 577.32 Mb on MorexV3. *Rph21* has been reported within the same genomic region (Sandhu et al., 2012), indicating a high probability that AGG-72 is *Rph21*. Clare et al., (2024) reported a projected MorexV3 interval of 569.19–587.48 Mb for *Rph21*, which partially overlaps the *RphAGG72* candidate region and suggests a high likelihood that AGG-72 carries *Rph21*. Very recently Azizinia et al., (in press, TAAG) identified and characterised a new resistance locus *RphCas* (in an Eastern European barley cultivar Caspian) which also mapped to 4H in the same physical region 576–583 Mb as *Rph21* and *RphAGG-72*. It will be worth undertaking allelic studies between these three loci and to understand their genetic relationship.

Collectively, these findings indicate that AGG-137 possesses two genes conferring resistance to *Ph* on chromosomes 3H and 5H. The locus on chromosome 3HS (*RphAGG-137*) maps to the same genomic region as reported for *Rph5*, *Rph6*, and *Rph7* but is distinct in its specificity and hence likely novel. The second locus, on chromosome 5HS, corresponds to *Rph2*. The wide spectrum resistance observed in AGG-137 in tests with multiple Australian *Ph* pathotypes indicates that the combination of these two resistance genes is effective despite virulence existing for each individually. *RphAGG-137* is therefore expected to have limited protective value if deployed singly, but could be useful in enhancing leaf rust resistance if deployed in strategic combinations of selected *Rph* genes.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest.

Author contribution

MM executed and lead the studies. DS conceptualized and supervised the studies. RFP provided all Australian pathotypes. MM and DS performed phenotyping and data analysis. MM, PMD, KF, and LZ

performed genotyping, recombination and/or tGBS analysis. DS and MM wrote first draft and all authors contributed to the manuscript and approved the submitted version.

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Figures

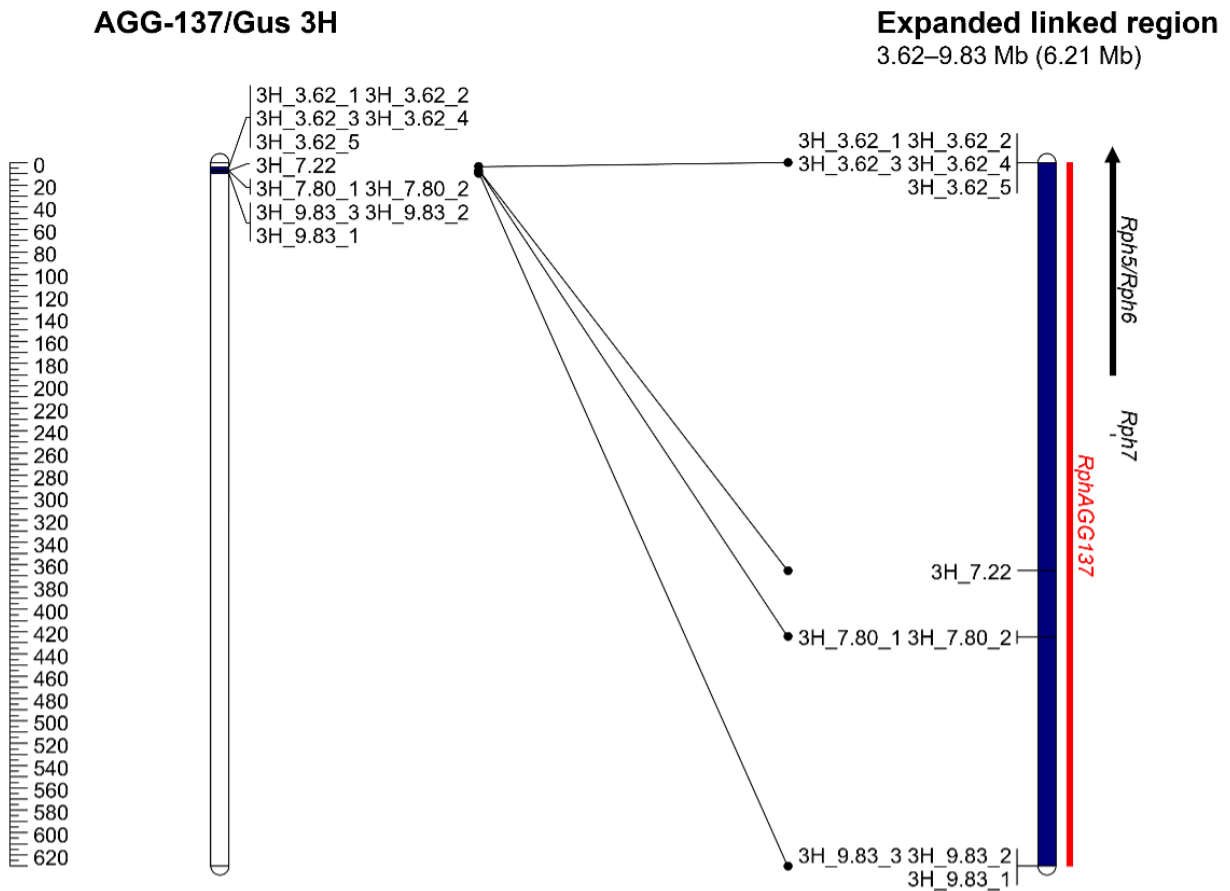


Figure 1

Candidate region identified in the AGG-137/Gus population. Physical positions are shown in Mb on the MorexV3 reference assembly. The full chromosome shows the region of high confidence markers on chromosome 3H. The expanded linked region shows the positions of the high confidence markers and *RphAGG-137*/*RphAGG137* in red. The published *Rph5/Rph6* interval (1.05–5.50 Mb) and *Rph7* locus (~6.03 Mb) are shown according to MorexV3 coordinates reported by Clare et al., (2024). Only the portion of the *Rph5/Rph6* interval overlapping the expanded *RphAGG137* candidate region is displayed, with the arrow indicating that the interval extends beyond the displayed region

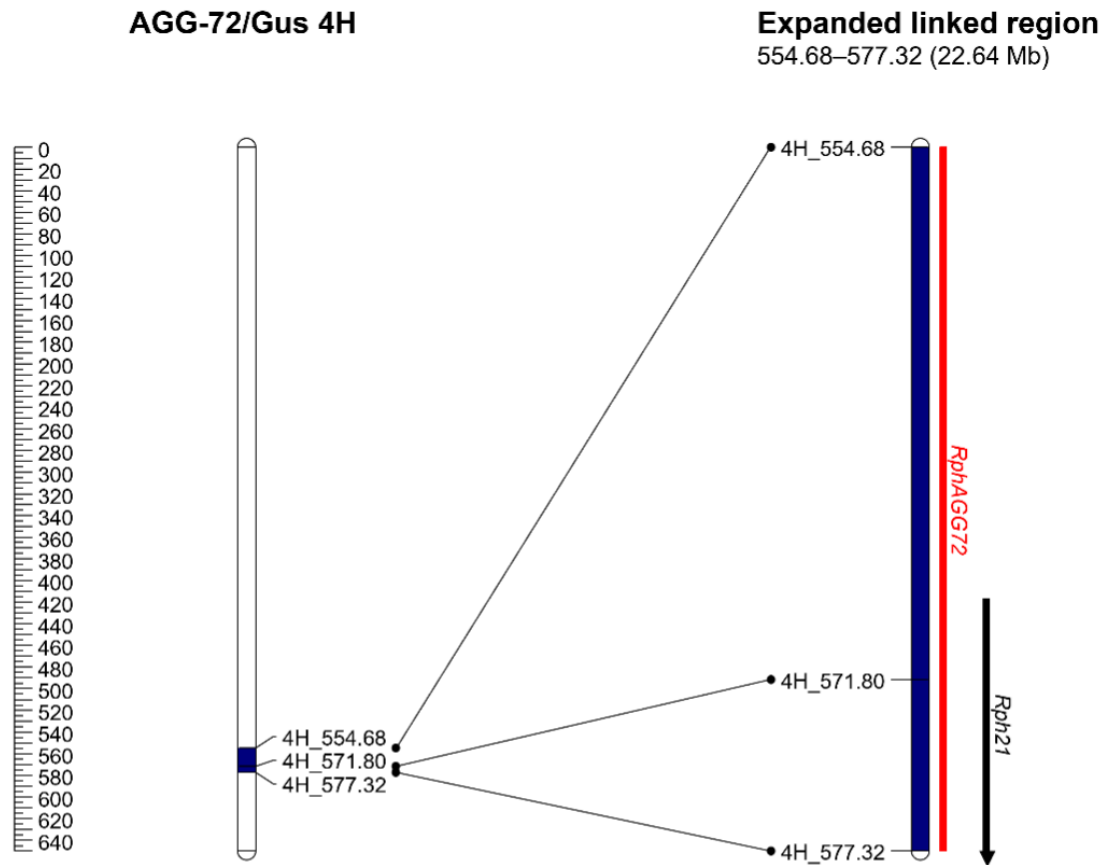


Figure 2

Candidate region identified in the AGG-72/Gus population. Physical positions are shown in Mb on the MorexV3 reference assembly. The full chromosome shows the region of high confidence markers on chromosome 4H. The expanded linked region *RphAGG-72* shows the positions of high confidence markers and *RphAGG72* in red. The published *Rph21* interval (569.19–587.48 Mb) is shown according to MorexV3 coordinates reported by Clare et al., (2024). Only the portion of the *Rph21* interval overlapping the expanded *RphAGG72* candidate region is displayed, with the arrow indicating that the interval extends beyond the displayed region

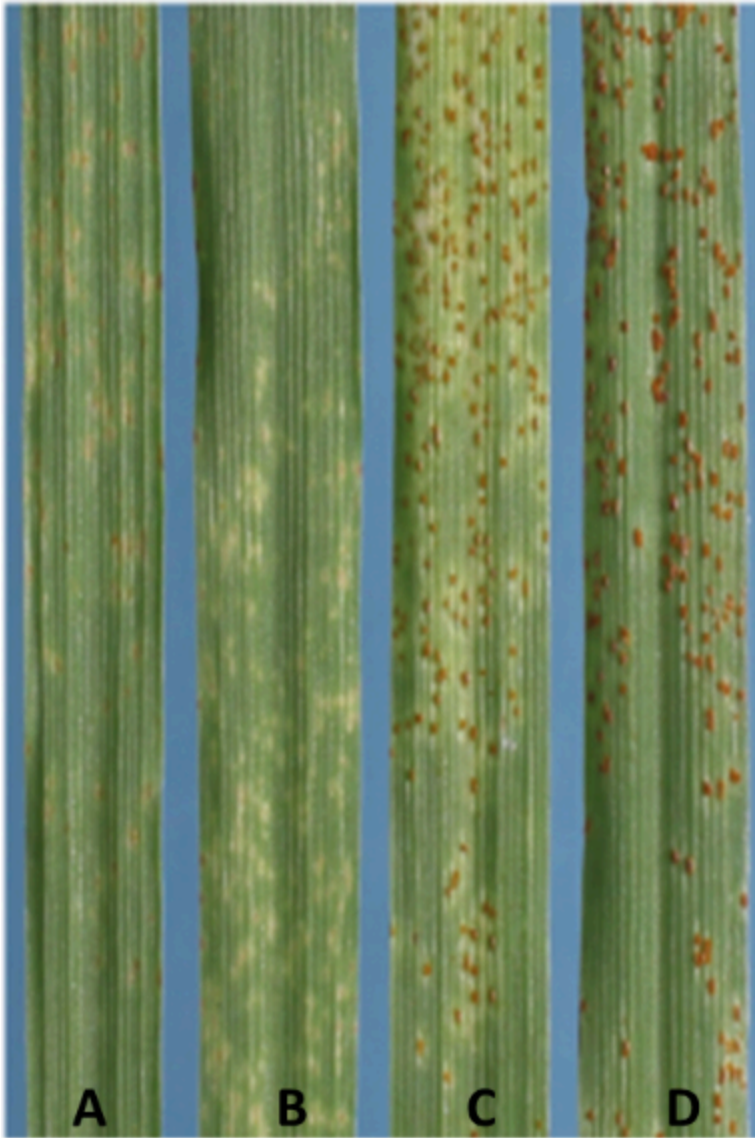


Figure 3

Seedling leaves of AGG-137 (from A to C) when inoculated with *P. hordeipathotypes* 220 P + +*Rph13*, 5457 P+ and 5477 P-) and Gus (D) inoculated with 5477 P-

Supplementary Files

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