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Unravelling Candidate Genes Associated With Blast Disease Resistance in an Elite Green Super Rice Varietal Panel Using Genome-Wide Association Study

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ABSTRACT

Rice blast disease, caused by the filamentous fungus *Magnaporthe oryzae*, significantly threatens global rice crops leading to yield losses worldwide. Given that existing resistance genes often fail to control rice blast due to the evolution of new virulent strains, identifying novel genes using modern breeding tools to enhance partial resistance is crucial for developing more durable and effective control measures. Here, we used a genome-wide association study (GWAS) with 2698 high-throughput single-nucleotide polymorphism (SNP) markers to analyse 134 rice cultivars from the Green Super Rice breeding programme against two virulent rice blast isolates, M101-1-2-9-1 (M101) and M64-1-3-9-1 (M64). GWAS identified 12 potential quantitative trait loci (QTLs) for blast resistance: five against M101 on chromosomes 1, 2, 3, 4 and 7 and seven against M64 on chromosomes 1, 4, 5, 6 and 12, with no QTL in common against both. Notably, *qM101_2* is within the 500 kb linkage disequilibrium (LD) block containing the known resistance gene *Pib* on chromosome 2, and *qM64_12.1* and *qM64_12.2* are within the *Pi-ta* and *Ptr* gene cluster on chromosome 12. The remaining nine QTLs represented novel blast disease resistance sources. The identified candidate genes, including those encoding nucleotide-binding site leucine-rich repeats domains, protein kinases, resistance gene analogues and pathogenesis-related proteins, may serve as a foundation for further studies to explore their potential role in enhancing disease resistance in rice.

1 | Introduction

Rice (*Oryza sativa*) is a major staple food crop and a vital nutrition source for approximately two-thirds of the global population (Liu et al. 2021). The cultivation and consumption of rice (both subspecies *indica* and *japonica*) are predominant in Asia, with the availability of irrigated and rainfed lands. *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*) is a fungal phytopathogen that causes rice blast disease, leading to global rice yield losses of about 10%–30% annually (Mandal et al. 2023; Wilson 2021). The spread of rice blast disease and the resulting losses among subsistence farmers have become alarming (Asibi, Chai, and Coulter 2019). Being a hemibiotroph, *M. oryzae* first develops a biotrophic interaction with the host cells by relying on the host for its growth and proliferation, then switches to necrotrophy by actively killing host cells (Martin-Urdiroz et al. 2016). Blast symptoms on rice leaves depend on environmental conditions, growth stage and resistance levels of host plants (TeBeest, Guerber, and Ditmore 2007). Susceptible cultivars typically display grey-green, water-soaked lesions with a darker green border that rapidly expands to several centimetres. Older lesions on susceptible cultivars often turn light tan with necrotic borders, whereas lesions on resistant cultivars remain small (1–2 mm) and exhibit a brown to dark brown colour (Jamaloddin et al. 2021; TeBeest, Guerber, and Ditmore 2007).

Blast disease management can be achieved through adopting or integrating resistant varieties, cultural practices, chemical and biological control methods, nutritional management and biotechnological techniques. Developing blast-resistant cultivars is the most effective and economical strategy for controlling this disease (Agbowuro et al. 2020). In recent decades, there has been a concerted effort to identify resistance (R) genes capable of combating *M. oryzae* isolates. The goal is to incorporate these genes into breeding pipelines to create durable and sustainable disease-resistant cultivars that can serve as alternatives to fungicides. R genes can either be complete or partial. Complete resistance to blast disease is regulated by the major R genes, which are qualitative, race-specific and prevent the completion of the life cycle of *M. oryzae* (Li et al. 2019). In contrast, partial resistance to rice blast disease is quantitative and durable due to the non-race-specific and polygenic traits (Li et al. 2019; Skamnioti and Gurr 2009), reducing the sporulation of the pathogen within a compatible interaction (Miah et al. 2013).

Research on blast resistance genetics began in the early 1920s when Sasaki observed varying responses of rice cultivars to different *M. oryzae* isolates (Sasaki 1922). Subsequent studies identified the first leaf-blast-resistance gene, *Pi-a*, from the *japonica* rice cultivar Aichi Asahi (Kiyosawa 1967). The first attempt to map a blast-resistance gene was done by Yu, Mackill, and Tanskley (1991), who used restriction fragment length polymorphism (RFLP) analysis to map two blast-resistance genes, *Pi-2(t)* and *Pi-4(t)*, using near-isogenic lines. Since the establishment of the initial genetic map of rice based on RFLP markers in 1988, a diverse array of genetic markers including random amplified polymorphic DNA (Monna et al. 1994), amplified fragment length polymorphism (Mackill et al. 1996), simple-sequence repeat (Chen et al. 1997), expressed sequence tag (Harushima et al. 1998), insertion and deletion (InDel; Lei et al. 2013) and single-nucleotide polymorphism (SNP; Liu et al. 2013), have been used for genetic mapping in rice. These efforts culminated in high-density genetic linkage maps of rice. The availability of complete genome sequences for rice subspecies *indica* and *japonica* has enabled researchers to exploit DNA polymorphisms, such as SNPs and InDels, for the fine-scale mapping of targeted genes (Kalia and Rathour 2019). Over the past decade, there has been a significant increase in studies aimed at identifying marker-trait associations (MTA) and related quantitative trait loci (QTLs) for blast resistance in rice. Since the first QTL analysis by Wang et al. (1994) numerous QTLs associated with blast disease resistance have been identified and documented with a diverse range of molecular markers, populations and environmental conditions. The genome-wide association studies (GWAS) approach has recently gained popularity for identifying genetic markers linked to QTLs or R genes conferring resistance to rice blast, thereby accelerating the cloning of rice blast resistance genes (Mgonja et al. 2016). To date, more than 230 candidate loci for blast resistance have been marked across 12 chromosomes in rice (Li et al. 2019; Mgonja et al. 2016; Yu, Ma, et al. 2022). These genes have been sourced from cultivated and wild rice (Younas et al. 2023) and function as protein kinases, transcription factors, ubiquitin-related proteins, phosphorylation-related proteins, DNA/ATP-binding proteins, oxidase/oxidoreductase and heat shock proteins. Recent advancements in molecular biology and genomics have facilitated the cloning of candidate resistance genes, opening up new possibilities for disease management (Ashkani et al. 2015; Yu, Ma, et al. 2022).

Over the past three decades, conventional breeding at the International Rice Research Institute (IRRI), Philippines, has developed elite rice cultivars with a wide array of disease-resistance genes known as IRRI-Green Super Rice (IRRI-GSR). Various methods, including the pedigree method, backcrossing, recurrent selection and mutation breeding are used in conventional breeding programmes (Bonman, Khush, and Nelson 1992; Miah et al. 2013). Conventional breeding programmes have introduced major genes for blast resistance, such as *Pib*, *Pita*, *Pia*, *Pi1*, *Pikh*, *Pi2* and *Pi4*, into rice cultivars (Koizumi 2007). While effective, conventional breeding methods have limitations, such as epistatic effects and the time required to develop resistant cultivars (Miah et al. 2013). Epistatic interactions between genes can obscure the expression of resistance traits, complicating the selection process (Divya et al. 2014). In addition, it typically takes 10 to 15 years to develop and release a new resistant cultivar, which may not keep pace with the evolving pathogen races with altered corresponding avirulence (AVR) genes (Majumder et al. 2021; Rosas et al. 2020).

Despite abundant information on resistance-related loci obtained through conventional breeding and contemporary approaches, blast epidemics remain uncontrolled due to host resistance breakdown and the emergence of new virulent pathotypes. Genetic events such as point mutations, insertion of transposable elements and gene deletions can result in the loss of function in AVR genes of *M. oryzae*, thereby affecting the effectiveness of the corresponding R genes in rice (Ning, Yunyu, and Aihong 2020). Therefore, identifying novel sources of resistance to rice blast remains essential, even with already identified R genes. In the present study, we used high-density SNP markers to conduct a GWAS for rice blast resistance on a diversity panel comprising 134 rice cultivars from the IRRI-GSR breeding programme. The cultivars were evaluated under controlled greenhouse conditions and challenged with two distinct isolates of *M. oryzae* native to the Philippines. Our objectives were to assess the potential of the IRRI-GSR breeding material for blast resistance and identify novel QTLs for breeding durable blast-resistant rice varieties.

2 | Materials and Methods

2.1 | Plant and Fungal Materials

One hundred and thirty-four rice accessions from the IRRI-GSR breeding programme were evaluated for resistance to rice blast disease under controlled conditions. Rice cultivars Lijiangxintuanheigu (LTH) and CO-39 served as susceptible checks. The two isolates used in this study, M101-1-2-9-1 (M101) and M64-1-3-9-1 (M64), are part of the standard differential blast isolates (SDBI) and were originally collected from Caliraya and Los Baños in the Laguna Province of Southern Luzon Island, Philippines (Hayashi 2005; Telebanco-Yanoria et al. 2008). Both isolates are known for their strong sporulating ability and have been extensively used in rice breeding programmes since the 1990s due to their consistent pathogenicity. The AVR gene profiles of the two isolates M64 and M101 demonstrate distinct patterns of interaction with known R genes in rice (Table S8; authors' unpublished data; Selisana et al. 2017). Both isolates carry *AvrPi9*, *AvrPik* and *AvrPita*, which support their incompatible reactions with rice genotypes harbouring the corresponding R genes *Pi9*, *Pik* and *Pita*. However, *AvrPiz-t* is present in M64 but absent in M101, while *AvrPib* is found in M101 but absent in M64. Neither isolate carries *AvrPii* or *AvrPia*, resulting in compatible reactions with rice lines with corresponding resistance genes (unpublished data).

2.2 | Experimental Setup and Inoculation

The seeds of the 134 rice accessions were oven-dried for 5 days at 50°C to break any residual seed dormancy. They were then pregerminated by placing 15 seeds in 90 mm plastic Petri dishes at 28°C for 2 days. After pregermination, the seeds were transferred to trays (35 × 27 × 12 cm) filled with sterilised Maahas clay loam, an isohyperthermic mixed-type tropical soil, and grown for 2 weeks under controlled conditions. A seedling stage blast screening experiment was arranged in an α-lattice design with two replications. Seedlings were planted in rectangular plastic trays (35 × 27 × 12 cm), each serving as a block and holding 300 seedlings (30 accessions with 10 seedlings per accession). Six trays were used per isolate, with three trays allocated to each replication. Optimum rice-growing conditions were maintained in the greenhouse: 29/21°C (day/night), 70% relative humidity, 16 h light/8 h dark photoperiod; and water saturation maintained at field capacity. At the 4- to 5-leaf stage, rice seedlings were inoculated with M101 and M64 isolates of *M. oryzae*. These isolates were cultured on prune agar (17 g agar, 1 g yeast, 5 g lactose monohydrate, pH 6.5) following the protocol described by Hayashi et al. (2009). The spore suspension (10^5 spores/mL) was sprayed onto rice using a mini-airbrush sprayer (Paasche Airbrush Company). A total of 30 mL of inoculum was used to spray each tray. Inoculated plants were incubated in the dark for 2 days under high humidity (more than 85%), after which they were returned to the greenhouse under optimum rice-growing conditions and monitored for disease progression over the next 6 days.

2.3 | Phenotypic Evaluation for Blast Resistance

Six days post-inoculation, the macroscopic lesions on the leaves overall were rated on a scale of 0 to 5 (0, no lesions; 1, uniform or scattered brown specks; 2, small lesions with distinct tan centres surrounded by a darker brown margin approximately 1 mm in diameter; 3, small eyespot lesions less than 1.5 mm in diameter surrounded by dark brown; 4, intermediate size eyespot lesions less than 2 mm in diameter and 5, large eyespot lesions more than 2 mm in diameter) as described by Fukuta, Cruz, and Kobayashi (2009). Disease score (SCO) ratings between 0 and 2 were associated with high resistance, a score below 3 (3−) was linked to moderate resistance and a score above 3 (3+) indicated moderately susceptible rice accessions. Scores between 4 and 5 were associated with high susceptibility, as described by Fukuta et al. (2009) with slight modifications. Apart from the qualitative disease score (SCO) trait, a total of eight quantitative traits including disease severity (SEV defined as the percentage of infected leaf area), number of lesions per cm^2 (L2), lesion length (maximum [MAX_L], minimum [MIN_L] and average [AV_L]) and lesion width (maximum [MAX_W], minimum [MIN_W] and average [AV_W]) were also measured for three leaves per accession per replicate using ImageJ software for both the isolates (Schneider, Rasband, and Eliceiri 2012).

2.4 | Statistical Analysis

The phenotype data were analysed using R (v. 4.4.1) (R Core Team 2023). The frequency distribution of the traits was plotted as a histogram using R. The normality of all phenotypic traits was assessed using the Shapiro–Wilk test in R. Analysis of variance (ANOVA) of disease traits was performed to determine the significance of differences among the rice genotypes (accessions), replicates and isolates using the 'lmer' function in R. The genotype, replicates and block were considered random factors in the model, as shown in Equation (1):

$$Y_{\mathrm{ijk}} = \mu + G_i + R_j + B_k + \varepsilon_{\mathrm{ijk}} \tag{1}$$

where phenotypic response (Y_{ijk}) is a function of the overall mean (μ), the *i*th genotype (G_i), the *j*th replication (R_j), the *k*th block (B_k) and the residual error ($\varepsilon_{\mathrm{ij}}$). Because the block effect was significant for only SEV and L2 traits for the M101 isolate, the 'block' term was removed from Equation (1), for other traits for each isolate. The variances obtained from the ANOVA were used to measure the broad-sense heritability (H^2). The best linear unbiased prediction (BLUP) values for all the traits were estimated for each isolate using the 'lmer' function from the lme4 package in R. These BLUP values were used for the GWAS analysis, as explained in the next section. Pearson's correlation analysis was performed to determine the relatedness between the two isolates using the 'corrplot' package in R.

2.5 | Genotyping, Linkage Disequilibrium and Population Structure

The 134 rice accessions used in this study were previously genotyped using the Cornell_7K_Array_Infinium_Rice (C7AIR) array, which includes 7098 SNP markers (Morales et al. 2020). Pairwise linkage disequilibrium (LD) between 7098 SNP markers within each chromosome and across the rice genome was calculated in the TASSEL v. 5.0 software package (Bradbury et al. 2007). The kinship matrix constructed in TASSEL helped determine the square of the allelic states (r^2) correlation between all SNP marker pairs on each chromosome for LD. The r^2 values were used to plot the LD decay over distance

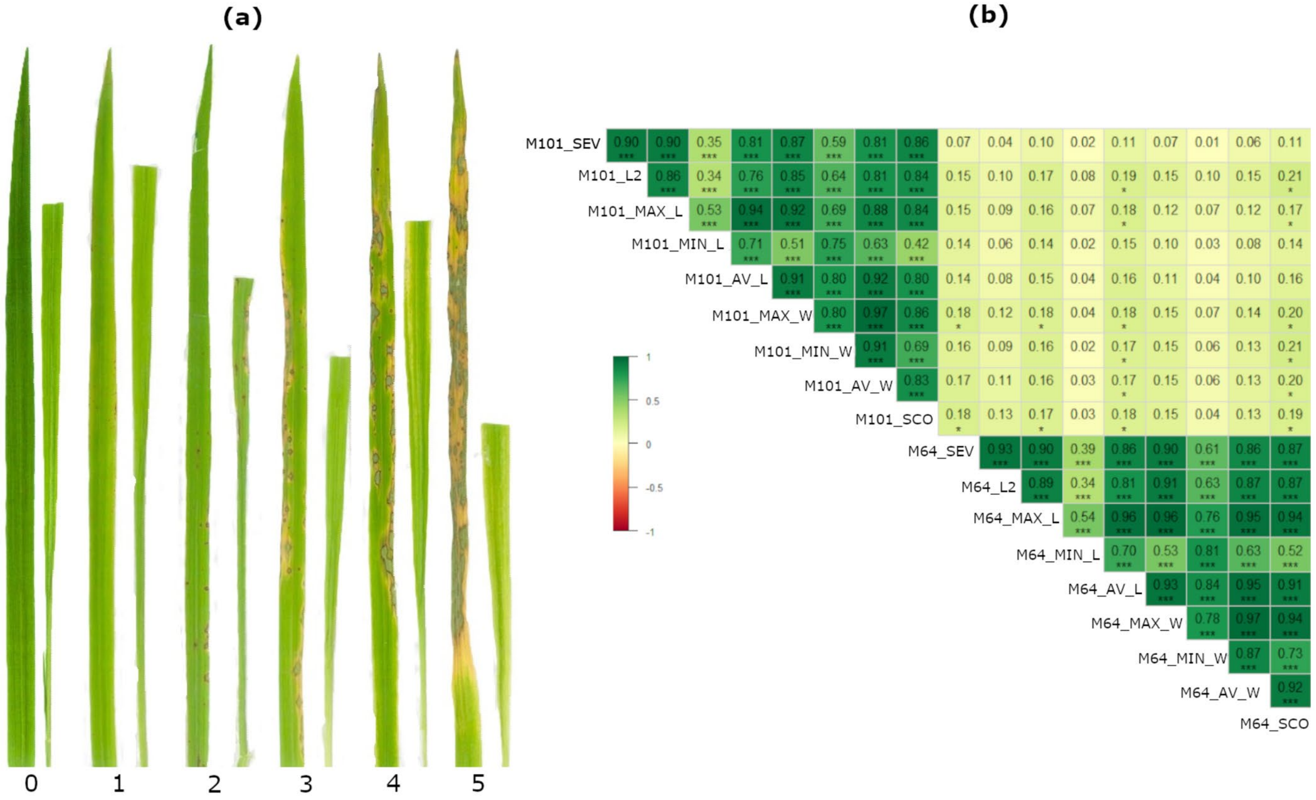

FIGURE 1 | (a) Rice leaves showing infection with rice blast pathogen, *Magnaporthe oryzae*, on a scale of 0 to 5 (0, no lesions; 1, uniform or scattered brown specks; 2, small lesions approximately 1 mm in diameter; 3, small eyespot lesions less than 1.5 mm in diameter; 4, intermediate size eyespot lesions less than 2 mm in diameter; 5, large eyespot lesions more than 2 mm in diameter); (b) Pearson's correlation matrix between traits for M101 and M64 isolates. The upper diagonal shows the correlation values between the traits along with their significance ($^*p<0.05$, $^{***}p<0.001$).

(bp) in R. The population structure of the 134 rice accessions was estimated through Bayesian analyses using STRUCTURE v. 2.3.4 (Pritchard, Stephens, and Donnelly 2000). The analyses comprised 100,000 Markov chain Monte Carlo iterations, preceded by a burn-in phase of 10,000 iterations for each run. The number of simulated clusters (K) ranged from 1 to 8, and 10 replicate runs were executed for each K value to estimate group assignment. To determine the optimal number of K, STRUCTURESELECTOR (https://lmme.ac.cn/StructureSelector/), a web-based software, was used (Li and Liu 2018). The selection process for the best K value was based on the method outlined by Evanno, Regnaut, and Goudet (2005), which aimed to maximise the rate of change in log-likelihood values (ΔK). Principal coordinate analysis further validated the population's genetic structure, which was conducted using GenAlEx v. 6.5, a genetic analysis in Excel (Peakall and Smouse 2006, 2012). A scree plot depicting the eigenvalues for each PC was also plotted using R.

2.6 | GWAS And Identification of Candidate Genes

The 7098 SNP genotypes of the 134 rice accessions were filtered with the criterion of minor allele frequency ≤ 5% and ≥ 25% missing data; thereby retaining 2698 SNPs for association studies. The BLUP estimates for all the traits for isolates M101 and M6 were used for the GWAS analysis. The Genomic Association and Prediction Integrated Tool (GAPIT v. 3.0), an R package, was used to perform GWAS (Wang and Zhang 2021). Five GWAS models were used in this analysis, namely, the general linear model (GLM), mixed linear model (MLM), multiple loci mixed model (MLMM), fixed and random model circulating probability unification (FarmCPU) and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK). MLM and GLM are single-locus test methods, while MLMM, FarmCPU and BLINK are multilocus test methods. Significant SNPs were filtered based on the Bonferroni correction threshold of $-\log_{10}(p)>4.73$ at $\alpha=0.05$ and visualised using Manhattan plots and QQ plots generated in R. To increase the confidence of the identified SNPs, a consensus-based approach was used, where an SNP identified by at least three out of five GWAS models was used for further analysis. Based on the half LD decay value, SNPs were classified into specific QTL regions. The coefficient of determination (adjusted R^2), representing the proportion of phenotypic variance explained (PVE) by the significant SNPs, was calculated by fitting an ordinary least square regression model in R, with the phenotype as the response variable and the significant marker as the independent variable. Candidate genes were identified using the reference IR64 genome on the Rice Gene Index (RGI; https://riceome.hzau.edu.cn), the first gene-based pangenome database for rice (Yu et al. 2023). The identification of candidate genes involved the 500 kb regions (250 kb upstream and 250 kb downstream) surrounding the peak SNPs associated with the QTL.

3 | Results

3.1 | Resistance Patterns, Heritability and Disease Trait Correlations

The disease score (SCO; on a scale of 0 to 5) for each isolate was based on a visual examination (Table S1, Figure 1a), and the disease reaction was categorised into four classes: resistant (R, between 0 and 2), moderately resistant (MR, 3−), moderately susceptible (MS, 3+) and susceptible (S, 4–5) (Table S2; Fukuta et al. 2009). For isolate M101-1-2-9-1 (M101), 92.5% of rice cultivars were resistant, and 6.7% of accessions were moderately resistant, while only one cultivar, IR 86522-29-10-1-1-1-1-1showed moderate susceptibility. No cultivars were classified as susceptible. In contrast, for isolate M64-1-3-9-1 (M64), 70.2% of accessions were resistant, 11.2% were moderately resistant, 11.2% were moderately susceptible and about 7.5% of rice cultivars were susceptible. The susceptible checks LTH and CO-39 showed high susceptibility having a score 5 on a scale of 0–5 (data not shown). Eight quantitative traits were also measured for isolates M101 and M64, namely, SEV, L2, MAX_L, MIN_L, AV_L, MAX_W, MIN_W and AV_W, and tabulated in Table S1. The frequency distribution of these quantitative traits confirmed a trend observed in the disease scores (Figure S1). The distribution for both isolates was right-skewed, indicating that most cultivars exhibited resistance, with fewer cultivars showing susceptibility. This right-skewed distribution aligns with the high percentage of resistant cultivars and suggests a non-normal phenotype data distribution. The Shapiro–Wilk normality test further supported this observation, showing significant deviations from normality ($p < 0.05$) (Table S3). ANOVA showed that the genotype effect was significant ($p < 0.001$) for all the traits associated with the M64 and M101 isolates (Table S3), except for the minimum length of lesions (MIN_L) and the average length of lesions (AV_L) of M101. Additionally, a significant block effect ($p < 0.05$) was observed for the traits SEV and L2 for isolate M101.

Broad-sense heritability (H^2) for all the traits of M101 ranged from 0.10 to 0.70, while the H^2 for isolate M64 ranged from 0.44 to 0.79 (Table S3). Notably, traits like SEV, L2, MAX_L, MAX_W and SCO had higher heritability values for both isolates. In contrast, the length and width of the lesions (minimum and average) yielded lower heritability estimates. A Pearson's correlation analysis was conducted for all the traits to compare the isolates and the traits under study (Figure 1b). The pairwise correlation of the nine phenotypic traits associated with the M64 isolate exhibited a very strong significant positive correlation ($0.80 < r < 1.00$) for most pairs. However, some trait pairs showed weak to moderate positive correlations ($0.20 < r < 0.79$). A similar correlation pattern was observed for the M101 isolate, with most traits showing strong positive correlations. However, when comparing the traits between the two isolates, we found significantly low positive to no correlation ($r = 0.17$–0.21).

3.2 | Population Structure and Linkage Disequilibrium

A total of 2698 SNP markers were retained after removing SNPs with ≥ 25% missing data and minor allele frequency (MAF) ≤ 5%, with an average of 224 markers/chromosome (Table S4). Bayesian analysis of population structure was performed on the genotypic data of 134 rice accessions and ΔK values were used to identify the presence of subpopulations. A plot of the number of clusters (K) versus ΔK revealed a peak at $K = 3$, indicating that the population is best described by three distinct subpopulations (Figure S2b). The three subpopulations were designated Pop1, Pop2 and Pop3, comprising 2, 16 and 64 genotypes, respectively, based on a probability value greater than 0.75 (Figure 2a). The remaining 52 genotypes were characterised as an admixture of the three subpopulations. The structure analysis results were supported by the principal coordinate analysis (PCoA), which showed that the three subpopulations were clustered separately, with the admixed genotypes clustered between them (Figure 2b). The first and second axes explained 10.01% and 6.37% of the total genetic variability, respectively. Additionally, a heat map of the kinship between the individuals (Figure 2c) and a scree plot (Figure S2a) corroborated the presence of the three subpopulations among the 134 rice accessions. A pairwise LD analysis of the SNPs was also conducted, estimating the mean LD decay over the physical distances (calculated as r^2) to be 513.3 kb, ranging from 384.7 kb for chromosome 1 to 733.5 kb for chromosome 7 (Figures 2d and S3, Table S4).

3.3 | GWAS And Identification of Candidate QTLs

Five GWAS models—GLM, MLM, MLMM, BLINK and FarmCPU—were used to identify SNPs linked to nine phenotypic traits for both the M101 and M64 isolates. We identified 201 SNPs of which 51 were unique. Among the 201, 62 and 139 SNPs were specific to isolates M101 and M64, respectively, all surpassing the Bonferroni correction threshold ($-\log_{10}(p) > 4.73$; Table S5). The distribution of these SNPs varied significantly across the different statistical models used. For isolate M101, GLM and MLM identified nine and four SNPs, respectively, while MLMM, BLINK and FarmCPU revealed 13, 20 and 16 SNPs, respectively. In contrast, isolate M64 showed a broader range of associations, with GLM identifying 55 SNPs, while 18, 15, 23 and 28 SNPs were identified through MLM, MLMM, BLINK and FarmCPU, respectively. The trait-wise analysis further illustrated that for isolate M101, most SNPs were associated with severity (17 SNPs), followed by maximum lesion length (13 SNPs). The minimum lesion width trait had the fewest associations, with only two SNPs. Contrastingly, in isolate M64, the highest number of SNPs was linked to severity (24 SNPs), with other traits showing substantial associations in a range of 11 to 21 SNPs. The least number of SNPs was associated with the trait L2 (eight SNPs). Chromosome-wise analysis revealed distinct patterns of SNPs for each isolate. For M101, SNPs were distributed with the highest numbers on Chr1 (10 SNPs), Chr2 (20 SNPs) and Chr7 (11 SNPs), while no SNPs were detected on Chr5, Chr9 and Chr10. On the other hand, M64 had a concentrated distribution with a notable accumulation on Chr12 (79 SNPs) and significant numbers on Chr4 (20 SNPs), while the remaining chromosomes exhibited fewer SNPs (ranging from 2 to 9), with no SNPs found on Chr10 and Chr11. A summary of the SNPs identified from GWAS analyses for isolates M101 and M64, covering different models, traits and chromosomes, is provided in Table S6.

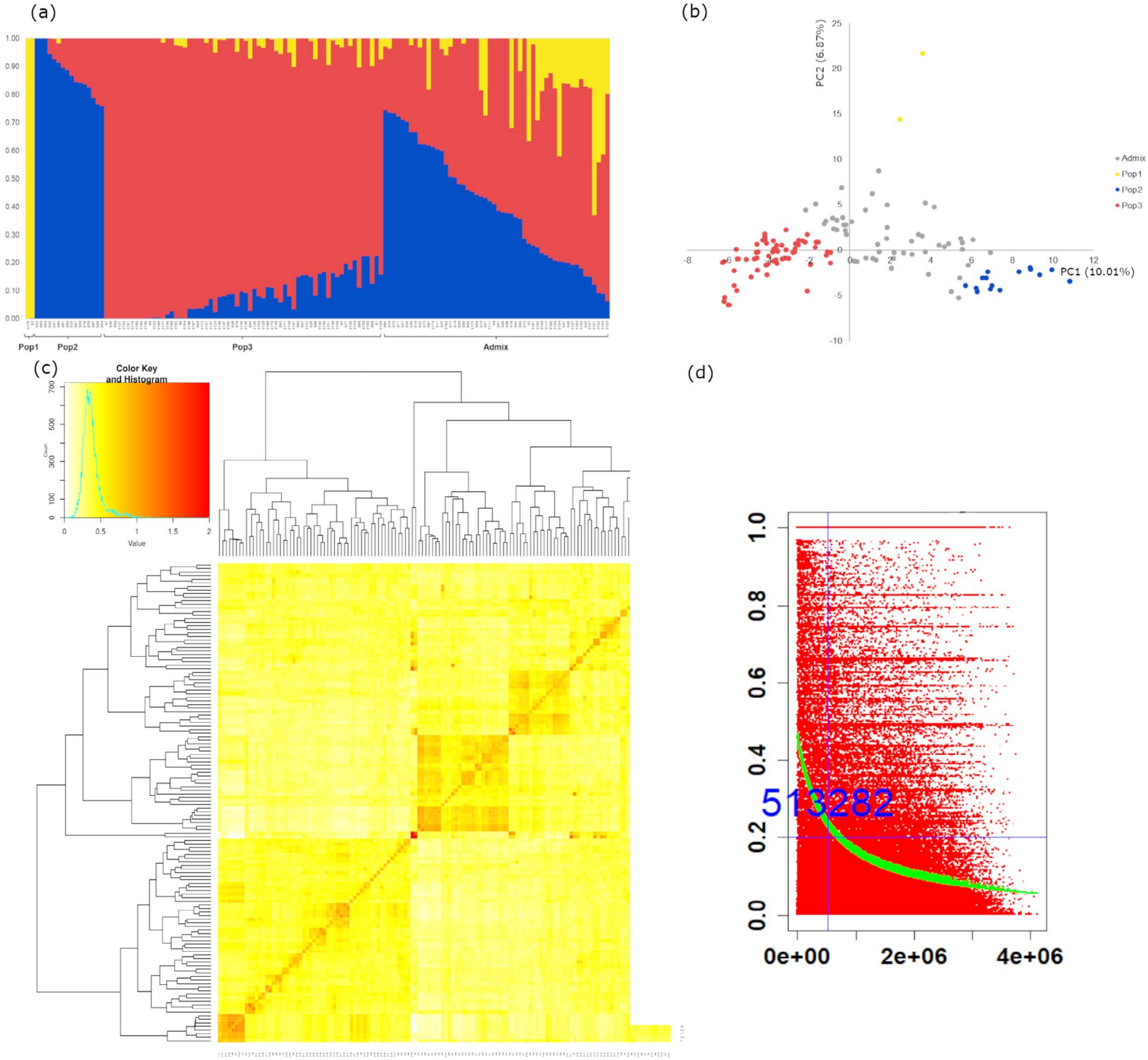

FIGURE 2 | Genetic diversity of the 134 IRRI-GSR accessions. (a) STRUCTURE bar plot showing the assignment of each rice accession at $K=3$ to the different genetic subpopulations identified (Pop1 in yellow, Pop2 in blue, Pop3 in red). (b) Two-dimensional principal coordinate analysis (PCoA) of the rice accessions depicting the three genetic subpopulations; accessions are colour-coded by STRUCTURE results at $K=3$ and admixed accessions are represented in grey dots. (c) Heat map of relatedness (kinship) showing the high (red) and low (yellow) degree of relatedness between the rice accessions. (d) Scatter plot of r^2 against genetic distance (bp) in rice accessions, showing the linkage disequilibrium (LD) decay across the whole rice genome. The blue vertical line indicates the point at which LD is reduced to 50% of its maximum value. The LD decay at the cut-off point is estimated at 513,282 bp.

We used a consensus-based approach, where an SNP was considered significant if identified by at least three out of five GWAS models. Thus, we refined our findings to 12 significant ones. Of these, five SNPs were specific to the M101 isolate with $-\log_{10}(p)$ of the peak SNP markers ranging between 4.76 and 9.61, and seven SNPs were specific to the M64 isolate, with $-\log_{10}(p)$ values ranging from 4.74 to 41.89 (Table 1, Figures 3 and S4). Among these, two SNPs for isolate M64 were associated with a single QTL *qM64_12.3*, while all the remaining 11 SNPs were associated with individual QTL. For the M101 isolate, QTLs were identified on chromosomes 1, 2, 3, 4 and 7, with 6.68% to 18.52% of PVE. For the M64 isolate, QTLs were localised on chromosomes 1, 4, 5, 6 and 12, with PVE ranging from 5.13% to 54.62%. The QTL on chromosome 6 demonstrated a maximum of 54.62% PVE against the M64 isolate for the minimum lesion length trait, which was the highest PVE value among all the 12 QTLs discovered for both the isolates (Table 1).

TABLE 1 | Summary of quantitative trait loci (QTLs) for resistance to rice blast isolates M101-1-2-9-1 (M101) and M64-1-3-9-1 (M64) identified by at least three genome-wide association study models using 134 IRRI-GSR accessions.

QTL	Chr	Position (bp)	SNP marker	Trait	Models	MAF	$-\log_{10}(p)$	% PVE
qM101_1	1	13,791,466	439,764	MAX_L, SCO, SEV	GLM, MLM, MLMM, BLINK, FarmCPU	0.060	4.86–8.31	14.50–15.39
qM101_2	2	34,919,721	SNP-2.34913851.	AV_L, AV_W, L2, MAX_L, MAX_W, MIN_W, SCO	GLM, MLM, MLMM, BLINK, FarmCPU	0.425	4.76–8.47	10.68–16.54
qM101_3	3	12,013,490	2,770,990	L2, MAX_L, SEV	GLM, MLM, MLMM, BLINK	0.496	5.08–9.61	12.25–18.52
qM101_4	4	648,669	3,598,944	MIN_L	GLM, MLM, MLMM, BLINK	0.056	4.84–6.70	14.78
qM101_7	7	744,502	id7000142	SCO, SEV	MLMM, BLINK, FarmCPU	0.203	5.64–6.61	6.68–6.91
qM64_1	1	12,486,799	389,017	MIN_L, MIN_W	GLM, MLM, BLINK, FarmCPU	0.064	4.76–5.07	18.74–20.27
qM64_4	4	17,467,268	4,296,140	AV_W, L2, MAX_L, MAX_W, SCO, SEV	GLM, MLM, MLMM, BLINK, FarmCPU	0.177	4.83–10.17	20.31–26.57
qM64_5	5	10,353,227	5,187,194	MIN_L	GLM, MLM, BLINK, FarmCPU	0.023	5.56–18.97	32.48
qM64_6	6	15,312,419	6,368,788	MIN_L, MIN_W	GLM, MLM, MLMM, BLINK, FarmCPU	0.496	5.21–41.89	16.83–54.62
qM64_12.1	12	10,724,877	SNP-12.10722289.	AV_L, AV_W, MAX_L, MAX_W, SCO, SEV	GLM, MLM, MLMM, BLINK, FarmCPU	0.383	4.80–5.78	25.16–36.87
qM64_12.2	12	11,257,069	c12p11257068	SEV	GLM, MLM, MLMM	0.297	4.87–5.49	32.08–37.30
qM64_12.3	12	12,388,405	id12004491	SEV	GLM, MLM, MLMM	0.342	4.74–11.17	28.65
		12,610,402	SNP-12.12607740	AV_L, MAX_L, MIN_L, SEV	GLM, MLM, BLINK, FarmCPU	0.357	4.75–8.51	5.13–29.53

Abbreviations: AV_L, average lesion length; AV_W, average lesion width; BLINK, Bayesian-information and linkage-disequilibrium iteratively nested keyway; Chr, chromosome; FarmCPU, fixed and random model circulating probability unification; GLM, general linear model; L2, number of lesions per cm^2; MAF, minor allele frequency; MAX_L, maximum lesion length; MAX_W, maximum lesion width; MIN_L, minimum lesion length; MLM, mixed linear model; MLMM, multiple loci mixed model; PVE, phenotypic variance explained; SCO, disease score; SEV, disease severity; SNP, single-nucleotide polymorphism.

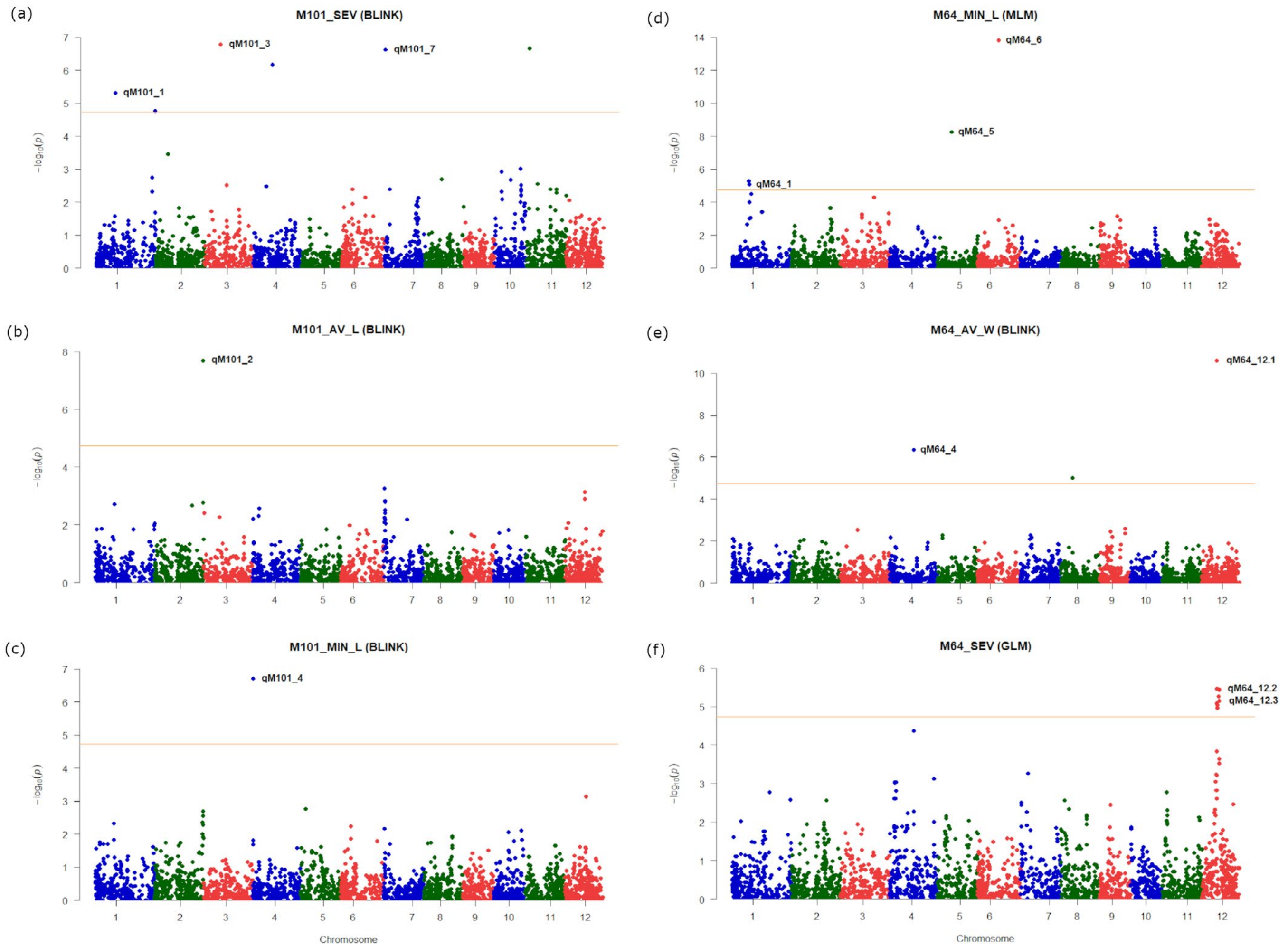

FIGURE 3 | Manhattan plots showing the significant single-nucleotide polymorphisms (SNPs) identified by genome-wide association studies for the resistance to the rice blast isolates M101 (a–c) and M64 (d–f). For M101, *qM101_1–4* and *qM101_7* were detected using the BLINK model for (a) severity (SEV), (b) average lesion length (AV_L) and (c) minimum lesion length (MIN_L). For M64, *qM64_1*, *qM64_4–6* and *qM64_12.1–3* were detected using (d) MLM (trait: MIN_L), (e) BLINK (trait: Average lesion width, AV_W) and (f) GLM (trait: SEV) models.

3.4 | Identification of Defence-Related Candidate Genes

Candidate genes linked to significant QTLs were identified within the 500 kb interval region using rice cultivar IR64 as the reference genome. For isolate M101, the number of genes identified 250 kb upstream and downstream ranged from 30 to 75 per QTL. For isolate M64, the identified genes ranged between 5 and 75 per QTL (Table S7). Among these genes, several were associated with plant defence, innate immunity or disease resistance, and these were compiled for each QTL, as shown in Table 2.

In the case of isolate M101, four out of five QTLs—*qM101_2*, *qM101_3*, *qM101_4* and *qM101_7*—located on chromosomes 2, 3, 4 and 7, respectively, included genes related to pathogenicity or plant disease resistance. For instance, *qM101_2* comprised genes such as pathogenesis-related protein PR-1, mitogen-activated protein kinase 4, genes from the ubiquitin-proteasome pathway and the putative F-box/FBD/LRR-repeat protein At3g49030. *qM101_3* and *qM101_4* contained putative disease resistance RPP13-like protein 1, while disease resistance protein RGA4 was linked to *qM101_4*. Additionally, *qM101_7* included genes such as putative disease resistance proteins RGA2 and RGA3, peroxidase 51, F-box/FBD/LRR-repeat protein At2g04230 and mitogen-activated protein kinase 5. Among all the QTLs discovered for M101, *qM101_1* did not contain any genes directly involved in pathogenesis or disease resistance. However, it comprised genes such as two-component response regulators (UniProtKB ID: Q5SML5) *ORR22* and *ORR23* and probable histidine kinase 2 (UniProtKB ID: Q5SML4), which play roles in the cytokinin signalling pathway; nuclear transcription factor Y subunit C-4 (UniProtKB ID: Q9FMV5), which is involved in the gibberellic acid-mediated signalling pathway; and dual specificity protein phosphatase *PHS1* (UniProtKB ID: Q75QN6), which has roles in the abscisic acid-activated signalling pathway.

For isolate M64, the QTLs identified include several genes across different chromosomes. The QTL *qM64_1*, located on chromosome 1, contained genes such as probable receptor-like protein (RLP) kinase, Pto-interacting protein 1 and CBL-interacting protein kinase 25. On chromosome 4, *qM64_4* encompassed a diverse set of genes, including disease resistance protein RGA2, RLP kinase HERK 1, ER lumen protein-retaining receptor B,

TABLE 2 | Candidate genes for resistance to rice blast isolates M101-1-2-9-1 (M101) and M64-1-3-9-1 (M64) identified by genome-wide association study using 134 IRRI-GSR accessions.

Isolate	QTL	Associated locus[a]	No. of genes[b]	Candidate gene	Position[c] (bp)	Gene length (bp)	Gene function[d]	Reference
M101-1-2-9-1	*qM101_1*	Chr1_13,791,466	30 (6)	OsIR64_01g0015400	13,701,076–13,703,316	2240	Two-component response regulator ORR22	—
				OsIR64_01g0015410	13,703,347–13,704,088	7435	Two-component response regulator ORR23	—
				OsIR64_01g0015420	13,704,654–13,708,447	3793	Probable histidine kinase 2	—
				OsIR64_01g0015530	13,956,545–13,957,963	1418	Nuclear transcription factor Y subunit C-4	—
				OsIR64_01g0015540	13,962,833–13,967,693	4860	Dual specificity protein phosphatase PHS1	—
				OsIR64_01g0015550	13,967,893–13,972,342	4449	Glycine-rich domain-containing protein 2	—
	qM101_2	Chr2_34,919,721	75 (13)	OsIR64_02g0035850	34,679,764–34,681,866	2102	Putative F-box/FBD/LRR-repeat protein At3g49030	—
				OsIR64_02g0035860	34,693,290–34,694,183	893	Pathogenesis-related protein PR-1	dos Santos and Franco (2023)
				OsIR64_02g0035870	34,694,814–34,695,350	536	Pathogenesis-related protein 1	dos Santos and Franco (2023)
				OsIR64_02g0035910	34,709,897–34,711,763	1866	Mitogen-activated protein kinase kinase 4	Asai et al. (2002)
				OsIR64_02g0035920	34,724,571–34,728,873	4302	E3 ubiquitin-protein ligase At4g11680	—
				OsIR64_02g0035940	34,743,156–34,750,979	7823	Diacylglycerol kinase 7	—
				OsIR64_02g0035970	34,762,821–34,765,103	2282	F-box protein SKIP8	—
				OsIR64_02g0035980	34,769,367–34,772,824	3457	Protein SUPPRESSOR OF MAX2 1	—

(Continues)

TABLE 2 | (Continued)

Isolate	QTL	Associated locus[a]	No. of genes[b]	Candidate gene	Position[c] (bp)	Gene length (bp)	Gene function[d]	Reference
				OsIR64_02g0036070	34,837,618–34,839,425	1807	Putative RING-H2 finger protein ATL49	—
				OsIR64_02g0036100	34,854,105–34,857,843	3738	Probable E3 ubiquitin-protein ligase XBOS32	—
				OsIR64_02g0036150	34,882,487–34,888,462	5975	Serine/threonine-protein kinase fray2	—
				OsIR64_02g0036400	35,049,308–35,055,721	6413	Ubiquitin carboxyl-terminal hydrolase 23	—
				OsIR64_02g0036550	35,160,193–35,160,969	776	Ethylene-responsive transcription factor ERF003	—
	qM101_3	Chr3_12,013,490	61 (8)	OsIR64_03g0015280	11,814,110–11,819,816	5706	CBL-interacting protein kinase 31	—
				OsIR64_03g0015350	11,898,104–11,901,709	3605	Guanylate kinase 2, chloroplastic/mitochondrial	—
				OsIR64_03g0015410	11,953,396–11,954,781	1385	Probable WRKY transcription factor 46	Hu, Dong, and Yu (2012), van Verk, Bol, and Linthorst (2011)
				OsIR64_03g0015590	12,057,661–12,059,198	1537	Non-specific lipid transfer protein GPI-anchored 2	Fahlberg et al. (2019)
				OsIR64_03g0015610	12,076,892–12,081,037	4145	Protein ETHYLENE-INSENSITIVE 3-like 1a	Mao et al. (2006)
				OsIR64_03g0015630	12,089,935–12,092,653	2718	Putative disease resistance RPP13-like protein 1	Bittner-Eddy et al. (2000)
				OsIR64_03g0015660	12,107,423–12,109,301	1878	E3 ubiquitin-protein ligase SIRP1	—
				OsIR64_03g0015670	12,110,871–12,114,759	3888	Pyruvate kinase, cytosolic isozyme	—

(Continues)

TABLE 2 | (Continued)

Isolate	QTL	Associated locus[a]	No. of genes[b]	Candidate gene	Position[c] (bp)	Gene length (bp)	Gene function[d]	Reference
	qM101_4	Chr4_648,669	35 (4)	OsIR64_04g0000400	560,023–575,573	15,550	Probable L-type lectin-domain containing receptor kinase S.5	—
				OsIR64_04g0000410	583,617–585,302	1685	L-type lectin-domain containing receptor kinase IV.2	—
				OsIR64_04g0000580	749,276–754,388	5112	Putative disease resistance RPP13-like protein 1	Bittner-Eddy et al. (2000)
				OsIR64_04g0000590	754,445–763,877	9432	Putative disease resistance protein RGA4	Okuyama et al. (2011)
	qM101_7	Chr7_744,502	63 (8)	OsIR64_07g0000890	720,849–730,564	9715	Suppressor of RPS4-RLD 1	—
				OsIR64_07g0001000	805,269–808,416	3147	Peroxidase 51	—
				OsIR64_07g0001010	808,852–811,739	2887	Receptor-like protein EIX2	Ron and Avni (2004)
				OsIR64_07g0001090	886,927-888,462	1535	Putative disease resistance protein RGA3	Xu et al. (2014)
				OsIR64_07g0001100	892,332–900,193	7861	Disease resistance protein RGA2	Xu et al. (2014)
				OsIR64_07g0001180	981,206–989,470	8264	F-box/FBD/LRR-repeat protein At2g04230	—
				OsIR64_07g0001200	989,747–996,023	6276	Mitogen-activated protein kinase kinase kinase 5	Asai et al. (2002)
M64-1-3-9-1	*qM64_1*	Chr1_12,486,799	51 (3)	OsIR64_01g0014360	12,379,252–12,384,569	5317	Probable receptor-like protein kinase At5g18500	—
				OsIR64_01g0014370	12,385,625–12,390,053	4428	Pto-interacting protein 1	Martin et al. (1993)
				OsIR64_01g0014510	12,558,864–12,559,169	305	CBL-interacting protein kinase 25	—

(Continues)

TABLE 2 | (Continued)

Isolate	QTL	Associated locus[a]	No. of genes[b]	Candidate gene	Position[c] (bp)	Gene length (bp)	Gene function[d]	Reference
	qM64_4	Chr4_17,467,268	45 (7)	OsIR64_04g0009530	17,227,676–17,232,908	5232	Disease resistance protein RGA2	Xu et al. (2014)
				OsIR64_04g0009540	17,245,355–17,246,629	1274	Receptor-like protein kinase HERK 1	—
				OsIR64_04g0009630	17,322,889–17,326,542	3653	ER lumen protein-retaining receptor B	—
				OsIR64_04g0009660	17,357,419–17,359,251	1832	Class V chitinase CHIT5	—
				OsIR64_04g0009890	17,630,113–17,631,600	1487	Wall-associated receptor kinase 2	—
				OsIR64_04g0009900	17,640,831–17,643,487	2656	Wall-associated receptor kinase-like 8	—
				OsIR64_04g0009930	17,709,702–17,714,716	5014	F-box/LRR-repeat protein 14	—
	qM64_5	Chr5_10,353,227	5 (1)	OsIR64_05g0009350	10,481,773–10,494,303	12,530	Probable LRR receptor-like serine/threonine-protein kinase At1g56130	—
	qM64_6	Chr6_15,312,419	11 (1)	OsIR64_06g0014880	15,272,771–15,274,578	1807	Receptor-like protein kinase 2	—
	qM64_12.3	Chr12_12,388,405_12,610,402	75 (2)	OsIR64_12g0010810	12,579,534–12,582,187	2653	Chloroplast stem-loop binding protein of 41 kDa b, chloroplastic	—
				OsIR64_12g0010960	12,784,838–12,785,818	980	Putative cyclin-dependent kinase F-2	—

[a] Chromosome_SNP position in bp.
[b] Total number of genes identified for a particular quantitative trait locus (QTL) in the 500 kb interval regions with the number of genes associated with disease resistance or plant defence responses in parentheses.
[c] Position identified in IR64 reference genome.
[d] Gene function was identified by rice gene index (RGI).

Class V chitinase CHIT5 and wall-associated receptor kinases. The *qM64_5* on chromosome 5 featured a probable leucine-rich repeats (LRR) receptor-like serine/threonine-protein kinase. The *qM64_6*, located on chromosome 6, included a RLP kinase. Finally, on chromosome 12, three QTLs were discovered, of which only one, *qM64_12.3*, contained a chloroplast stem-loop binding protein, which may have a potential role in defence against pathogens.

4 | Discussion

Global food security faces escalating challenges amidst climate change, particularly in rice production, with the rise of several pathogen populations including the devastating *M. oryzae*, causing rice blast disease (Liu et al. 2021; Singh and Maurya 2021). Integrating broad-spectrum and durable resistance has become a primary concern among breeders to manage rice blast (Devanna et al. 2022; Ning, Yunyu, and Aihong 2020). Traditional breeding methods, such as stacking multiple R genes, often encounter issues with rapid resistance breakdown over time or limited efficacy of similar-function genes (Ning, Yunyu, and Aihong 2020; Wing, Purugganan, and Zhang 2018). However, the GSR breeding strategy uses advanced genomic technologies and diverse QTLs for stress tolerance, aiming to offer more, durable, sustained and robust protection against rice blast disease (Ali et al. 2021; Wing, Purugganan, and Zhang 2018; Yu et al. 2020; Yu, Ali, et al. 2022). To build on these advantages and further optimise breeding for improved resistance to rice blast disease, this study assessed nine blast disease-related traits across 134 IRRI-GSR accessions under controlled conditions for two virulent *M. oryzae* isolates, M101-1-2-9-1 and M64-1-3-9-1.

The blast isolates exhibit differential reactions to known R genes, demonstrating unique compatibility and allele-specific resistance mechanisms. M64 shows incompatible reactions with *Pita*, *Piz-t* and *Pib* resistance loci, suggesting the absence of the corresponding AVR genes (Telebanco-Yanoria et al. 2008). In addition, M64 is virulent to most rice accessions with the Ptr_b allele, while resistant to those carrying the Ptr_a and Ptr_c alleles, emphasising the allele-specific resistance at *Ptr* locus (Greenwood et al. 2024). In contrast, M101 overcomes *Pita2/AvrPita2*- mediated resistance but shows an incompatible reaction with *Piz* and compatible reaction with *Piz-5* (Meng, Zhang, and Jin 2020; Telebanco-Yanoria et al. 2008).

Our study observed significant variation in disease response among the rice accessions when exposed to the two isolates (Table S2). A predominant tendency towards resistance was noticeable, with only a few accessions showing susceptibility, suggesting that the studied population is largely resistant and the genetic factors conferring resistance are already prevalent (Figure S1). Moderate to relatively high estimates of broad-sense heritability ($H^2 > 0.50$) for most traits suggest that the diversity panel offers strong potential as a genetic resource for breeding rice for blast tolerance, with many traits likely to respond to selection and be heritable (Table S3, de Visser et al. 2003). However, a few traits showed lower heritability, indicating a greater influence of environmental or other non-genetic factors on their variation. Additionally, the correlation analysis revealed strong positive correlations among traits for each isolate, suggesting interconnectedness probably influenced by similar genetic or environmental factors within each isolate (Figure 1b). However, weak correlations between traits across the two isolates highlight distinct responses to M101 and M64, reflecting differences in their pathogenicity mechanisms and host interactions (Meghanathan 2016).

The GWAS identified 12 QTLs associated with M101 and M64 across nine blast disease-related traits, with five QTLs linked to M101 (chromosomes 1, 2, 3, 4 and 7) and seven to M64 (chromosomes 1, 4, 5, 6 and 12) (Tables 1, S5 and S6). Chromosome 1 contains well-characterised blast resistance genes, such as *Pi37* (Os01g0781700; Lin et al. 2007), *Pi64* (Os01g0781200; Ma et al. 2015) and *Pish* (Os01g0782100; Takahashi et al. 2010), which are part of a resistance gene cluster on rice chromosome 1. Additionally, *Pit* (RAP ID: Os01g0149500) from Indonesian rice variety Tjahaja is another CC-NBS-LRR on this chromosome (Hayashi and Yoshida 2009). However, identified QTLs *qM64_1* (at 1,24,86,799 bp on the Reference genome: IR64) and *qM101_1* (13,791,466 bp) are distant from these known genes, such as *Pit* (2,681,220–2,686,364 bp) and *Pi37* cluster (33,098,082–33,145,541 bp). This suggests that *qM64_1* and *qM101_1* may represent novel loci associated with rice blast resistance. Another QTL, *qM101_2*, was detected on chromosome 2 at position 34,919,721 bp. Chromosome 2 consists of the dominant blast resistance gene *Pib* (Os02g0818500), which confers high resistance to many Japanese *M. oryzae* isolates (Wang et al. 1999). *Pib* is mapped to the distal long arm of chromosome 2 (35,113,637–35,118,769 bp) and encodes a nucleotide-binding site leucine-rich repeats (NBS-LRR or NLRs) protein. Interestingly, the SNP for *qM101_2* is just 1.93 kb from *Pib* and falls within its 500 kb LD block, suggesting that *qM101_2* could either be *Pib*, a regulatory element influencing its expression, or a novel resistance gene in the same region. Furthermore, *AvrPib* gene was found to be present in M101, supporting the hypothesis that *qM101_2* may be associated with the *Pib* locus or a closely linked resistance mechanism (authors' unpublished data).

Chromosome 4 hosts several key blast-resistance genes, including *Pi21* (Os04g0401000) at position 19,83,5206 bp, which provides durable, non-race-specific resistance through *pi21* loss-of-function mutation (Fukuoka et al. 2009), and *Pi63* (Os04g0620950), also known as *Pikahei-1(t)*, at position 31,55,1754 bp, known for strong field resistance in upland rice (Xu et al. 2014). Here, we identified two significant QTLs, *qM101_4* and *qM64_4*, at 6,48,669 bp and 1,74,67,268 bp, respectively, on chromosome 4. These QTLs are outside the regions of *Pi21* and *Pi63*, suggesting they may represent novel genetic loci or regulatory elements contributing to blast resistance. On chromosome 6, *qM64_6* was identified at 15,312,419 bp in response to the M64 isolate, distant from known blast resistance gene clusters such as *Pi9* (2,410,176–2,418,566 bp; Qu et al. 2006), *Pi2/Piz-t/Pi50* (10,387,793–10,390,465 bp; Su et al. 2015; Zhou et al. 2006), *Pi25* (13,054,818–13,058,041 bp; Chen et al. 2011; Shang et al. 2009) and *Pi-d2* (17,160,333–17,164,820 bp; Chen et al. 2006). This suggests that *qM64_6* may represent a novel resistance locus.

On chromosome 12, a key resistance gene cluster includes *Pi-ta* (Os12g0281300), *Pi-ta2* (Os12g0281300) and *Ptr* (Os12g0285100) and has been crucial in controlling rice blast in *indica* varieties globally since the 1960s. *Pi-ta* encodes a predicted 92 kDa cytoplasmic receptor with a nucleotide-binding site (NBS; Bryan et al. 2000), while *Ptr* is critical for *Pi-ta*/*Pi-ta2* resistance (Zhao et al. 2018). Three QTLs are located on chromosome 12: *qM64_12.1* (10,724,877 bp), *qM64_12.2* (11,257,069 bp) and *qM64_12.3* (12,388,405; 12,610,402 bp). *qM64_12.1* lies 118 kb from *Pi-ta* and 97 kb from *Ptr*, and *qM64_12.2* is 443 kb from *Ptr*, suggesting potential linkage to this resistance gene cluster. However, they have not been previously reported in the literature, and further studies are required to confirm their role in rice blast resistance. In contrast, *qM64_12.3* does not co-localise with these genes, indicating it may represent a novel source of resistance. Additionally, *qM101_3* on chromosome 3, *qM64_5* on chromosome 5 and *qM101_7* on chromosome 7 appear to be new resistance loci, as no resistance genes have been previously cloned or reported in these chromosomes (Ning, Yunyu, and Aihong 2020).

Following the identification of these QTLs, we examined candidate genes within a 500 kb interval to uncover potential mechanisms underlying rice blast resistance (Tables 2 and S7). Pathogenesis-related (PR) proteins, such as PR-1, accumulate in infected tissues and are linked to disease resistance (Vidhyasekaran 2007). PR-1, present in the apoplast during plant–pathogen interactions, exhibits antifungal activity through cell wall thickening, preventing pathogen invasion (dos Santos and Franco 2023; Vidhyasekaran 2007). Our study associates PR-1 (UniProtKB IDs: Q40374, Q05968) with QTL *qM101_2*, suggesting a likely involvement in resistance against M101. We also identified plant resistance gene analogues (RGAs) as key components of the defence mechanism. RGA4, linked to *qM101_4*, and RGA2, associated with *qM64_4* on chromosome 4, along with RGA2 and RGA3 linked to *qM101_7* on chromosome 7, are noteworthy. RGAs are classified into two groups: NBS-LRRs and pattern recognition receptors (PRRs). NLRs, involved in pathogen recognition and defence activation, include toll/interleukin receptor (TIR)-NBS-LRR (TNL) and non-TIR/coiled-coil-NBS-LRR (CNL) proteins (Sekhwal et al. 2015; Tirnaz et al. 2020). In our study, TNLs linked to *qM101_2*, *qM101_7* and *qM64_4* were associated with F-box/FBD/LRR-repeat proteins (UniProtKB ID: Q9SMU0), At2g04230 (UniProtKB ID: Q6NKX3) and F-box/LRR-repeat protein 14 (UniProtKB ID: Q3EC97), respectively. For CNL proteins, we identified a putative RPP13-like protein 1 (UniProtKB ID: Q9LRR4) linked to *qM101_3* and *qM101_4*, known for its role in downy mildew resistance against *Peronospora parasitica* in *Arabidopsis thaliana* (Bittner-Eddy et al. 2000).

Among the PRRs, resistance genes encoding receptor-like kinases (RLKs) and RLPs play a significant role in plant defence mechanisms (Sekhwal et al. 2015; Tirnaz et al. 2020; Vidhyasekaran 2007). In our study, we discovered several PRRs linked to QTLs. For instance, a serine/threonine-protein kinase fray2 (UniProtKB ID: Q9M0E5) was associated with *qM101_2*, and *Pto*-interacting protein 1 (UniProtKB ID: Q41328) associated with *qM64_1*, a kinase lacking the LRR domain but involved in pathogen signalling. The tomato *Pto* gene encodes a serine/threonine kinase that lacks an LRR domain but plays a critical role in pathogen recognition (Martin et al. 1993). We also identified other protein kinases linked to all QTLs except *qM101_1*, which are vital for pathogen recognition and signalling (Vidhyasekaran 2007). *qM101_2* and *qM101_7* were linked to mitogen-activated protein kinase 4 (UniProtKB ID: O80397) and 5 (UniProtKB ID: Q9C5H5), respectively, which are involved in the defence response to fungi, mitogen-activated protein kinase (MAPK) signalling cascade and PRR signalling pathways. Moreover, receptor-like cytoplasmic kinases (RLCKs), crucial mediators within these pathways, were identified (Asai et al. 2002; Cortaga et al. 2022; Sekhwal et al. 2015). RLP EIX2 (UniProtKB ID: Q6JN46), associated with *qM101_7*, functions as a receptor for the fungal elicitor ethylene-inducing xylanase in tomatoes (Ron and Avni 2004). These findings highlight the critical role of RLCKs and related proteins in pathogen recognition, reinforcing the potential of the identified QTLs as novel sources of resistance to *M. oryzae* in rice.

Beyond NLRs and PRRs, we identified several other proteins involved in plant defence, particularly within the ubiquitin-proteasome pathway (Table S7). F-box proteins, involved in phytohormone signalling and immune responses, effector-triggered immunity and pattern-triggered immunity, along with RING proteins and other E3 ligases being linked to SNPs, underscores the involvement of the ubiquitin-proteasome machinery in plant defence against rice blast (Abd-Hamid et al. 2020; Devoto, Muskett, and Shirasu 2003; Saxena, Negi, and Sharma 2023; Wang et al. 2022). In addition to the ubiquitin-proteasome system, we also identified a probable WRKY transcription factor 46 (UniProtKB ID: Q9SKD9) and non-specific lipid transfer protein GPI-anchored 2 (UniProtKB ID: Q9LZH5) linked to *qM101_3*. WRKY64 activates salicylic acid biosynthesis for systemic acquired resistance (van Verk, Bol, and Linthorst 2011) and mediates basal resistance against *Pseudomonas syringae* (Hu, Dong, and Yu 2012). LTPs contribute to defence against pathogens and abiotic stresses, with roles in non-host resistance against powdery mildew in *A. thaliana* (Fahlberg et al. 2019; Finkina et al. 2016; Gao et al. 2022; Saxena et al. 2023). We also identified ethylene-responsive transcription factor ERF003 (UniProtKB ID: Q94AW5) and protein ETHYLENE-INSENSITIVE 3-like 1a (UniProtKB ID: Q10M41) linked to *qM101_2* and *qM101_3*, respectively, as well as peroxidase 51 (UniProtKB ID: Q9SZE7), associated with *qM101_7*, all of which are involved in plant defence responses to fungi and environmental stresses such as wounding and pathogen attack (Broekaert et al. 2006; Mao et al. 2006).

In summary, this study assessed phenotypic and genotypic variations in 134 IRRI-GSR breeding panel accessions for resistance to two virulent *M. oryzae* isolates, revealing key QTLs and candidate genes associated with blast resistance on chromosomes 1, 2, 3, 4, 5, 6, 7 and 12, some of which represent novel resistance loci. The predominantly resistant population exhibited significant variation in resistance traits and high heritability, underscoring the potential of these genetic resources for rice blast tolerance. Identified candidate genes included NLRs, PRRs, protein kinases and components of the ubiquitin-proteasome system, suggesting diverse defence mechanisms. These findings provide a solid foundation for future research and the development of rice varieties with broad-spectrum and durable resistance. Future work will focus on the molecular confirmation of *qM101_2* as *Pib* by analysing resistant accessions using gene-specific primers for *Pib*. Additionally, further investigation will be conducted to validate the roles of *qM64_12.1* and *qM64_12.2* within the *Pi-ta* and *Ptr* gene cluster, as well as to characterise the remaining novel QTLs as potential new sources of resistance (Yadav et al. 2017). These efforts will enhance the identification and deployment of durable resistance genes in rice breeding programmes.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

This research article offers extensive data supporting its conclusions, presented in figures, tables, and additional supporting information (tables and figures).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.