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Genetics and Genomics of Resistance

Identification of a Major Rice Blast Quantitative Trait Locus Containing *Pita/Pi39(t)/Ptr* in U.S. Black Hull Awn Weedy Rice

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Abstract

Rice blast, caused by the ascomycete fungus *Magnaporthe oryzae*, is one of the most problematic diseases for rice production, threatening global food security. Genetic resistance to some *M. oryzae* races can be achieved using major resistance genes that recognize their corresponding fungal avirulence genes. Weedy rice, a close relative of cultivated rice that competes with the crop, has evolved unique genetic mechanisms to resist *M. oryzae* infections; thus, weedy rice can serve as an excellent resource for blast control. In this study, we assessed disease scores of 183 F_5 and F_6 recombinant inbred lines (RILs) derived from a weedy rice × crop biparental mapping population and their parental lines, a Black Hull Awn weedy rice strain (PI 653413, RR14) and the aus-196 rice variety, using four distinct common U.S. blast races (IB33, IG1, IE1K, and IC17) under greenhouse conditions. All the parental lines were resistant to all blast races; however, RILs showed a wide degree of variation in resistance. Genotyping-by-sequencing of the RIL population and parents generated 1,498 single-nucleotide polymorphisms, which were used to construct a linkage map, and quantitative trait locus (QTL) mapping of blast resistance was performed using r/qtl. A single major blast resistance QTL on chromosome 12 was mapped to the *Pi-ta/Pi39(t)/Ptr* locus. Identification of *Pi-ta/Pi-39(t)/Ptr* as the key contributor to blast resistance in weedy rice provides insight into the evolution and adaptation of weedy rice and can aid in the development of blast-resistant rice varieties through marker-assisted selection.

Keywords: linkage map, *Magnaporthe oryzae*, mapping population, QTL, resistance genes, weedy rice

Rice (*Oryza sativa*) is a crucial food crop for at least 50% of the world's population (Sharma et al. 2012). Rice production is threatened by both abiotic and biotic stresses, and blast disease caused by the ascomycete fungal pathogen *Magnaporthe oryzae* (anamorph, *Pyricularia oryzae*) is the major biotic stress limiting rice production globally (Ashkani et al. 2015). Annual yield losses due to blast disease are estimated to be 10 to 30%, which translates to approximately 157 million tons of lost rice production worldwide (Deng et al. 2017; Liu et al. 2013). Rice blast management through application of fungicides has been employed (Kongcharoen et al. 2020; Pak et al. 2017); however, fungicide application has negative environmental effects, and in some cases, fungicide resistance within pathogen populations has been reported (Deising et al. 2008; Kim and Kim 2009).

The incorporation of rice blast resistance genes into cultivars therefore remains an important means of disease control. Resistance to blast is mediated by major and/or minor genes. Complete resistance is controlled by major resistance (*R*) genes and is specific to blast races containing the corresponding avirulence (*AVR*) genes. To date, more than 100 blast *R* genes have been identified clustered on different rice chromosomes (Srivastava et al. 2017). On chromosome 6, more than 11 *R* genes, including *Piz*, *Pizt*, *Pigm*, *Pi2*, *Pi9*, *Pi22*, *Pi25*, *Pi26*, *Pi40*, *Pi42*, and *Pi50*, have been identified that show broad-spectrum resistance, with *Pi9* (Qu et al. 2006), *Pi50* (Su et al. 2015), *Pigm* (Deng et al. 2017), *Pi2*, and *Pizt* (Zhou et al. 2006) cloned. On chromosome 11, cloned genes include *Pik* (Zhai et al. 2011), *Pi47* (Huang et al. 2011), *Pikm* (Ashikawa et al. 2008), and *Pik-h* (Sharma et al. 2010). Chromosome 12 accounts for more than 20 *R* genes, several of which have been cloned; these include *Pi-ta* (Bryan et al. 2000), *Ptr* (Zhao et al. 2018) that is *Pi-ta2* (Meng et al. 2020), *Pi19(t)* (Hayashi et al. 1998), and *Pi48* (Huang et al. 2011).

Most blast *R* genes function as ligand receptors and are categorized based on their conserved domains, which include the following: leucine-rich repeat (LRR), nucleotide-binding site (NBS), toll-interleukin receptor, coiled-coil, and protein kinase transmembrane receptor (Kourelis and van der Hoorn 2018). The *R* gene *Ptr* that encodes a protein with 4 armadillo repeats whose function is independent of two NLR proteins, *Pi-ta* and *Pi-39(t)*, has been predicted to play an important role in *Pi-ta/Pi-39(t)* disease resistance (Zhao et al. 2018).

M. oryzae is known to undergo rapid evolution due to the movement of transposable elements within its genome; this reorganization of repetitive sequences results in the loss of *AVR* gene recognition by major *R* genes and leads to resistance breakdown. Multiple incidences of blast overcoming plant immunity have been reported. In Yunnan, China, mutations in the *AVR-Pi9* gene resulted in the breakdown of resistance in *Pi9*-containing rice varieties (Lu et al. 2023). In Arkansas, United States, Zhou et al. (2007) reported that *M. oryzae* isolates with loss of *AVR-Pita* and insertion of a transposable element in *AVR-Pita* were virulent on rice varieties containing *Pi-ta/Ptr*. Wang et al. (2017) surveyed the U.S. blast population and

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found that with time, the blast fungus developed resistance to major *R* genes and became more pathogenic. To date, major *R* genes, including *Pi-ta/Pi39(t)/Ptr* (*Pi-ta2*), *Pi-z*, and *Pi-ks/h*, have successfully been deployed individually or in combination to provide robust blast resistance against U.S. blast races IB1, IA45, IB49, IH1, IC1, IB54, ID1, IC17, and IE1 (Fjellstrom et al. 2004; Jia et al. 2004; Lee et al. 2009; Zhao et al. 2018). A highly virulent blast race, IB33, without *AVR-Pita* was identified in the laboratory to overcome resistance mediated by *Pi-ta/Pi39(t)/Ptr* (Lee et al. 2009).

Durable blast resistance, mediated by minor *R* genes found on different rice chromosomes (Hu et al. 2008; Poland et al. 2009; Wisser et al. 2005), offers non-race-specific polygenic partial/quantitative resistance. It is therefore much harder for the pathogen to simultaneously evolve resistance against multiple different minor resistance loci compared with a single major *R* gene. Continued discovery of minor or quantitative trait loci (QTLs) involved in blast resistance is an important goal for rice breeding. QTLs involved in blast resistance have been identified from resistant rice cultivars that are inbred lines (Chen et al. 2018; Fang et al. 2016; He et al. 2017; Jia et al. 2022; Liu et al. 2008), back-crossed lines (Jiang et al. 2020; Ying et al. 2022), and doubled haploids (Kongprakhon et al. 2010; Lee et al. 2016).

Weedy rice (*Oryza sativa* L.) is an invasive agricultural pest that vigorously invades rice fields in rice-growing regions. It is found in rice fields worldwide, and previous studies have documented multiple independent evolutionary origins of this weed through de-domestication (endoferalization) from diverse rice cultivars worldwide, as well as from crop hybridization with wild rice and/or between existing weed populations (Grimm et al. 2020; Li et al. 2004; Reagon et al. 2010; Vigueira et al. 2019). Weedy rice aggressively competes with cultivated rice, decreasing yields and at the same time contaminating the rice harvests (Burgos et al. 2006; Cao et al. 2006). Despite posing a serious threat to rice production, weedy rice has unique competitive abilities and associated traits that could be useful for rice breeding, including resistance to rice blast disease (Lee et al. 2011; Liu et al. 2015; Zhao et al. 2022). The Ptr^{BHA} allele in a weed genotype is a new resistant haplotype effective in preventing infections by IB33, a virulent race without *AVR-Pita* (Zhao et al. 2022). *R* genes present in weedy rice could therefore act as a novel source of blast resistance (Osakina and Jia 2023).

The objectives of this study were to construct a linkage map and identify blast resistance loci and candidate genes in another weedy rice genotype using a mapping population derived from the cross from a distinct weedy rice genotype with an aus genotype.

Materials and Methods

Plant materials

A weed × crop recombinant inbred line (RIL) population was created by crossing one *aus* rice variety (aus-196) with a U.S. weedy rice genotype, the Black Hull Awn (BHA) weedy rice (PI 653413, RR14) (Jia and Gealy 2018). Crosses were carried out at the U.S. Department of Agriculture-Agricultural Research Service (USDA) Dale Bumpers National Rice Research Center in Stuttgart, Arkansas. aus-196 is a breeding/research accession developed in Bangladesh and obtained from the USDA plant germplasm collection (GSOR 301597). RR14 (AR-1994-10A) weedy rice was collected in 1994 in Prairie County, Arkansas, and maintained by the Dale Bumpers National Rice Research Center (Jia and Gealy 2018). This BHA/aus mapping population, named "MH" and consisting of 185 individuals, was advanced through selfing under greenhouse conditions using single seed descent to F_5 seeds and then advanced one additional generation under field conditions in 2022. RILs at the F_6 generation were used for genotyping and phenotypic evaluation.

Phenotypic evaluation of blast disease reaction

Parental plants together with RILs were grown in the greenhouse at the USDA-ARS Dale Bumpers National Rice Research Center in Stuttgart, Arkansas, at a temperature of 25 to 30°C in plastic trays of 54 by 2 by 6 cm in size. Five biological replications of 15 plants per RIL were used for blast inoculations to generate disease phenotypic data. A total of four fungal blast races, IC17 (+*AVR-Pita*), IG1 (+*AVR-Pita*), IB33 (−*AVR-Pita*), and IE1K (−*AVR-Pita*), were used for disease evaluation in the greenhouse. Spore production, suspension, inoculation, and evaluation were performed as described by Wang et al. (2015). Briefly, blast isolates were grown on oatmeal agar plates (BD Difco, Franklin Lakes, NJ, U.S.A.) at 25°C for 9 days under dark and light conditions. Spores were then washed with a 0.25% gelatin solution (Fisher Scientific, Waltham, MA, U.S.A.) and filtered with four layers of cheesecloth. The final spore suspension was adjusted to a concentration of 1×10^5 spores/ml. Three-week-old rice seedlings (three- to four-leaf stage) (Chen et al. 2019) were sprayed with 20 ml of spore suspension per tray in a plastic bag. The sprayed plants were sealed in the inoculated bags and maintained at a temperature of 25°C and high humidity conditions (>90% relative humidity) for 24 h. Plants were then transferred to the greenhouse at 25 to 28°C, with 80% relative humidity and 12 h each of daylight and darkness. Disease evaluation was determined 7 days after inoculation using a rating scale of 0 to 5 described previously (Liu et al. 2015; Zhao et al. 2022), where individual scores represent pathotypes of rice varieties observed under field conditions as 0 = no visible lesions, 1 = a few small-point lesions, 2 = lesion size less than 2 mm and without fungal mass, 3 = 10% of leaf area with lesions bigger than 2 mm, 4 = greater than 10% and less than 50% of the leaf area with lesions bigger than 3 mm, and 5 = lesions greater than 50% of the leaf area. One numeric score was assigned to 15 seedlings for each replicate. For QTL mapping, three consistent replicates out of the five blast inoculations replications performed of each blast race were selected and used to generate average disease scores (Supplementary Table S1). This average disease score appeared in integer form, similar to previously reported studies (Jia et al. 2022; Jiang et al. 2020; Liu et al. 2015; Zhao et al. 2018).

Genotypic data analysis of genotyping-by-sequencing

Plants for genotyping were grown in the greenhouse of Washington University in St. Louis in the in the summer of 2022 under suitable greenhouse lighting and temperature conditions. After seedlings reached the three-leaf stage, 0.05 g of tissue was collected, frozen with liquid nitrogen, and ground to a powder. DNA extraction for each plant was performed using a modified CTAB protocol (Gross et al. 2009). Library preparation for genotyping-by-sequencing was performed by digesting with the ApeKI enzyme following established protocols (Wright et al. 2018). After library preparation, sample quality was checked using a Qubit 2.0 Fluorometer. Samples were sent to Novogene (Sacramento, CA, U.S.A.) for paired-end short-read sequencing on the HiSeq platform. Sequence data in fastq format were de-multiplexed, trimmed, aligned, and converted to bam files using the SABRE, cutadapt, bwa, and samtools packages, respectively, as part of the fastgbs_V2 pipeline (Torkamaneh et al. 2020). Single-nucleotide polymorphisms were called using the mpileup function of the bcftools software package. Filtering was then performed with the latest vcftools software package (Danecek et al. 2011) with the following flags: –remove-indels, –max-alleles 2, –hwe 0.0000000001, –maf 0.05, and –max-missing 0.80. Genotype data are shown in Supplementary Table S1.

Linkage map construction, QTL mapping, and candidate gene identification

Genomic data for five RIL lines (181, 182, 183, 184, and 185) were removed because of genotypic duplication. Linkage map construction was performed using r/qtl (Supplementary Fig. S1). Prior to linkage mapping, all heterozygous sites were set to missing, as

is common for RIL populations. Marker order and chromosome assignment were based on the physical location of the single-nucleotide polymorphisms in the MSU Release 7 rice reference genome (https://rice.uga.edu/). To identify potentially misplaced markers, pairwise recombination frequencies were calculated using the est.rf (https://rdrr.io/cran/qtl/man/est.rf.html) function, and markers that showed detectable recombination frequencies with markers on other chromosomes were manually identified and removed. Map distances for the remaining 1,498 markers were then calculated using the Kosambi function with a genotyping error rate of 0.01.

QTL mapping was performed in R with the R/qtl package (Broman et al. 2003) using the Haley–Knott function (Haley and Knott 1992). QTLs were considered significant if they surpassed a 0.05 threshold calculated from 1,000 permutations. Percent variance explained and effect size were calculated using the fitqtl (https://www.rdocumentation.org/packages/qtl/versions/1.66/topics/fitqtl) function, and 1-limit of detection (LOD) confidence intervals were calculated using the calcCis function in r/qtlTools (Lovell 2017). Naming of QTLs was based on the procedure stipulated by the gene nomenclature system for rice (McCouch 2008).

Candidate genes within the QTLs identified were predicted based on the output of gene prediction on rice genome ANNOTATION PROJECT MSU-Rice genome annotation (Osa1) Release 7 (https://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/).

Fine mapping of *qBLAST12*

To fine map the *qBLAST12* identified in this study, markers HJ17-11 and HJ17-13 previously used to distinguish *Pita* and *Ptr*, respectively, in Dee Geo Woo Gen from a BHA strain of weedy rice (Zhao et al. 2022) were used detect the presence of *Pita* and *Ptr* in parental lines and 118 RILs. Approximately 20 seeds of parental lines and RILs were germinated in Petri dishes at 32°C for 5 to 7 days, and DNA was isolated from five seedlings using the rapid extraction method developed by Xin et al. (2003) with a slight modification. Briefly, seedlings were transferred to a 96-well plate, and 70 µl of 100 mM NaOH and 2% Tween 20 was added. The plate was then incubated at 95°C for 10 min, followed by neutralization of the samples with 70 µl of 100 mM Tris-HCl (pH 8.0) and 2 mM EDTA. The DNA was then diluted with one part DNA and three parts TE (pH 8.0), and 4 µl of the DNA was utilized for PCR. PCR amplification and allele calling were performed using the method in Liu et al. (2008) with slight modifications. Briefly, PCR amplifications were performed in 25-µl reaction volumes consisting of 4 µl of DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X100, 250 µM dNTPs, 3.0 mM $MgCl_2$, 0.1% bovine serum albumin, 1% PVP40, 300 nM of each primer, and 1 unit of Taq DNA polymerase (Monserate Biotech, San Diego, CA, U.S.A.). Reverse primers were unlabeled, and the forward primers were labeled with HEX (Integrated DNA Technologies, Coralville, IA, U.S.A.). DNA was amplified in an Eppendorf Mastercycler (Eppendorf, Enfield, CT, U.S.A.) under the following conditions: initial denaturation at 94°C for 5 min, then 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a 5-min final extension at 72°C. The DNA was diluted 100× for HJ17-11 and 40× for HJ17-13, pooled along with the size standard 400HD ROX (Applied Biosystems, Waltham, MA, U.S.A.), and electrophoresed on an ABI 3730 XL (Applied Biosystems) following the manufacturer's standard procedure. The alleles were sized using GeneMapper v.3.7 (https://genemapper.software.informer.com/3.7/; Applied Biosystems).

TABLE 1. Blast disease scores of parental lines

Parental line	Blast race[a]			
	IB33	IE1K	IC17	IG1
aus-196	2	0	2	0
RR14	2	2	0	2

[a] Blast disease score of parental lines on a scale of 0 to 5, where 0 to 2 represents resistance and 3 to 5 represents susceptibility. At least three blast evaluation repeats of each score were performed using blast races IB33, IE1K, IC17, and IG1.

Results

Blast reaction of parental lines and RILs

Blast inoculation assays performed in the greenhouse showed that all blast isolates used in this study were avirulent on both parental lines, RR14 (BHA weedy rice) and aus-196 (*aus* cultivated rice) (Table 1; Fig. 1). For mapping purposes, we initially targeted blast races that are virulent and avirulent on each parental line. However, after several inoculations, we found that most of the blast races were avirulent, with very few being pathogenic, thus indicating that both the parental lines could contain blast resistance QTLs. For RILs, there was great variation in disease reaction (Supplementary Table S2). For instance, with blast race IB33, 133 RILs were resistant, whereas 50 were susceptible; for IG1, 158 RILs were resistant, and 25 were susceptible; and IC17 showed 59 RILs being susceptible and 124 RILs being resistant. Finally, for blast race IE1K, the majority of RILs (172 RILs) exhibited resistance, and only 11 were susceptible (Fig. 2). The greater variation in disease reactions observed among RILs compared with their parents indicates that the two parental lines could be harboring multiple blast resistance QTLs, making the mapping population ideal for novel blast *R* gene discovery.

Mapping of blast resistance QTLs

Following removal of five RILs that were genotypic duplicates, the remaining 178 RILs were used to create a linkage map comprising 1,498 single-nucleotide polymorphisms spanning the 12 rice chromosomes (Supplementary Fig. S1), with an average distance of 1.13 cM between markers (Supplementary Table S3). Two major blast resistance QTLs, both derived from the weedy rice parent, were mapped on chromosome 12 (Table 2; Fig. 3A and B). A similar QTL was also mapped previously in BHA weedy rice using blast race IB33 within this region (Liu et al. 2015). One of these, the major QTL *qBLAST12*, was located between the markers S12_7748829 and S12_15804848 and accounted for 21.12% of disease resistance variance against the blast race IB33 (Table 2). Similarly, *qBLAST12* was identified using blast race IC17, with an LOD score of 7.791926 and percent variance explained of 18.26% (Table 2). The last major QTL, *qBLAST12-1*, was detected by blast race IG1 with flanking

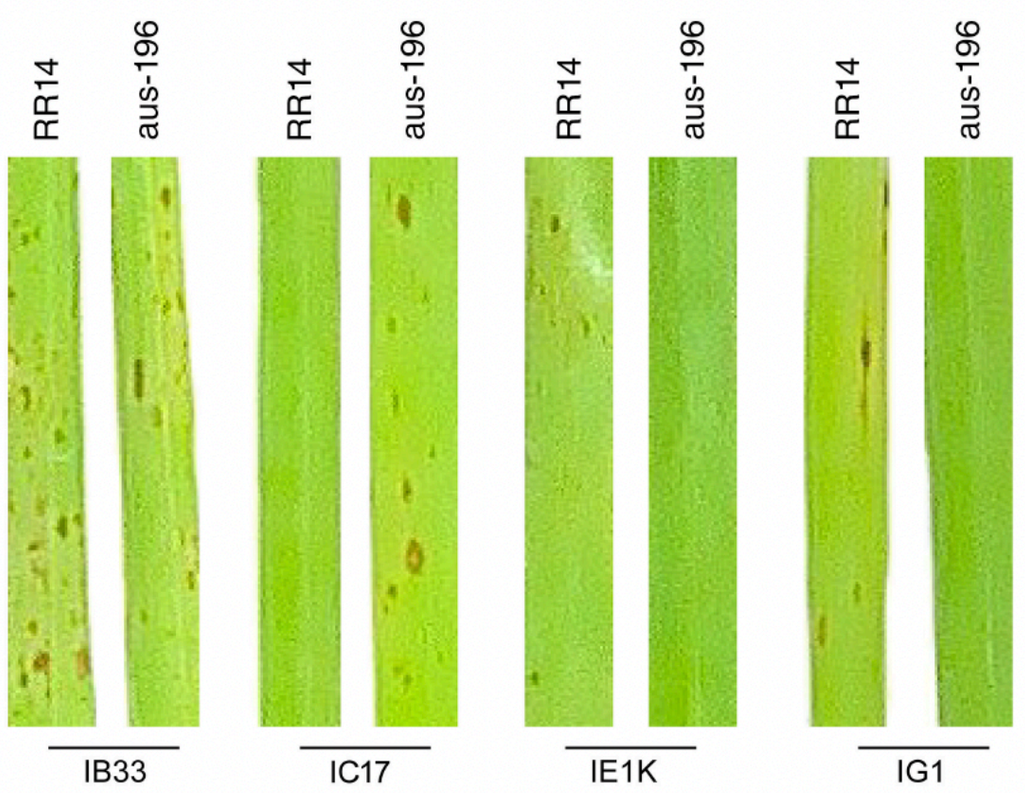

Fig. 1. Rice blast disease development on parental lines aus-196 and Black Hull Awn weedy rice (RR14) inoculated with four blast races: IB33, IC17, IE1K, and IG1. Leaf images were taken at 7 days postinoculation.

markers S12_6229842 and S12_15804848, a region that encompasses and extends beyond that of *qBLAST12*. The LOD score and the percent phenotypic variation of *qBLAST12-1* were 5.090207 and 12.33895, respectively (Table 2). The wider chromosomal span of *qBLAST12-1* compared with *qBLAST12* could potentially reflect the involvement of an additional minor-effect locus against blast race IG1 that differs from *qBLAST12*, although it could also be a simple statistical artifact of the linkage mapping. The r/qtl mapping program used in this study was unable to detect minor-effect QTLs, despite the presence of peaks (Fig. 3A); this could be because most of the peaks had an LOD score of less than 3.

Candidate genes

The locations of the candidate genes within *qBLAST12* and *qBLAST12-1* were predicted on the MSU Rice Genome Annotation Project Release 7 (https://rice.uga.edu). The exact location of a gene was determined by searching the region within the QTL using its corresponding flanking molecular markers on the output of gene prediction by rice Genome Annotation Project MSU-Rice genome annotation (Osa1) Release 7 (https://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/). All predicted blast *R* candidate genes are listed in Table 3. They include three major blast *R* genes detected within *qBLAST12*, with two, *Pi-ta* (*LOC_Os12g18360*) (Bryan et al. 2000) and *Pi-39(t)* (*LOC_Os12g18374*) (Liu et al. 2007), NB-ARC (nucleotide-binding domain, Apaf-1, R proteins, and CED-4) encoding proteins and the third being a typical armadillo repeat-containing *Ptr* gene (*LOC_Os12g18729*) (Zhao et al. 2018). Other putative *R* genes within *qBLAST12* include four NB-ARC encoding proteins (*LOC_Os12g17090*, *LOC_Os12g17410*, *LOC_Os12g25170*, and *LOC_Os12g17430*) (Yu et al. 2018), stripe rust resistance protein Yr10 (*LOC_Os12g17490*), and two LRRs (*LOC_Os12g17420* and *LOC_Os12g17340*). In *qBLAST12-1*, in addition to 10 putative *R* genes identified within *qBLAST12*, nine extra disease *R* genes were detected. They include five Verticillium wilt disease *R* genes (*LOC_Os12g11680*, *LOC_Os12g11720*, *LOC_Os12g11860*, *LOC_Os12g12010*, and *LOC_Os12g12120*). Other alleles within *qBLAST12-1* are disease resistance RPM1 protein *LOC_Os12g14330*, NBS-LRR disease resistance *LOC_Os12g13550*, disease resistance protein SlVe2 *LOC_Os12g11930*, and disease resistance family protein *LOC_Os12g11940* (Table 3).

The major QTL identified is indistinguishable from the *Ptr* allele

Previously, Liu et al. (2015) identified QTL *qBR12.3* in BHA weedy rice (RR20) using blast race IB33. QTL *qBR12.3* was later fine mapped at the *Ptr* locus (Zhao et al. 2022). In our present study, QTL *qBLAST12* was mapped to the *Pita/Ptr* region by blast race IB33 and contributed to 21.1152% disease resistance. To determine if *qBLAST12* is due to *Pi-ta* or *Ptr*, molecular markers HJ17-11 for *Pi-ta* and HJ17-13 for *Ptr* (Zhao et al. 2022) were used to detect the presence of *Pi-ta* and *Ptr* in parental lines and RILs. Amplicons of 143 and 144 bp indicating *Pi-ta* alleles were detected in aus-196 and BHA weedy rice (RR14) parental lines, respectively, whereas 241 bp indicating the *Ptr* allele was only detected in RR14 (Supplementary Table S4). The majority of the RIL population (58 out of 79; 73%) that showed resistance to blast races IB33, IC17, and

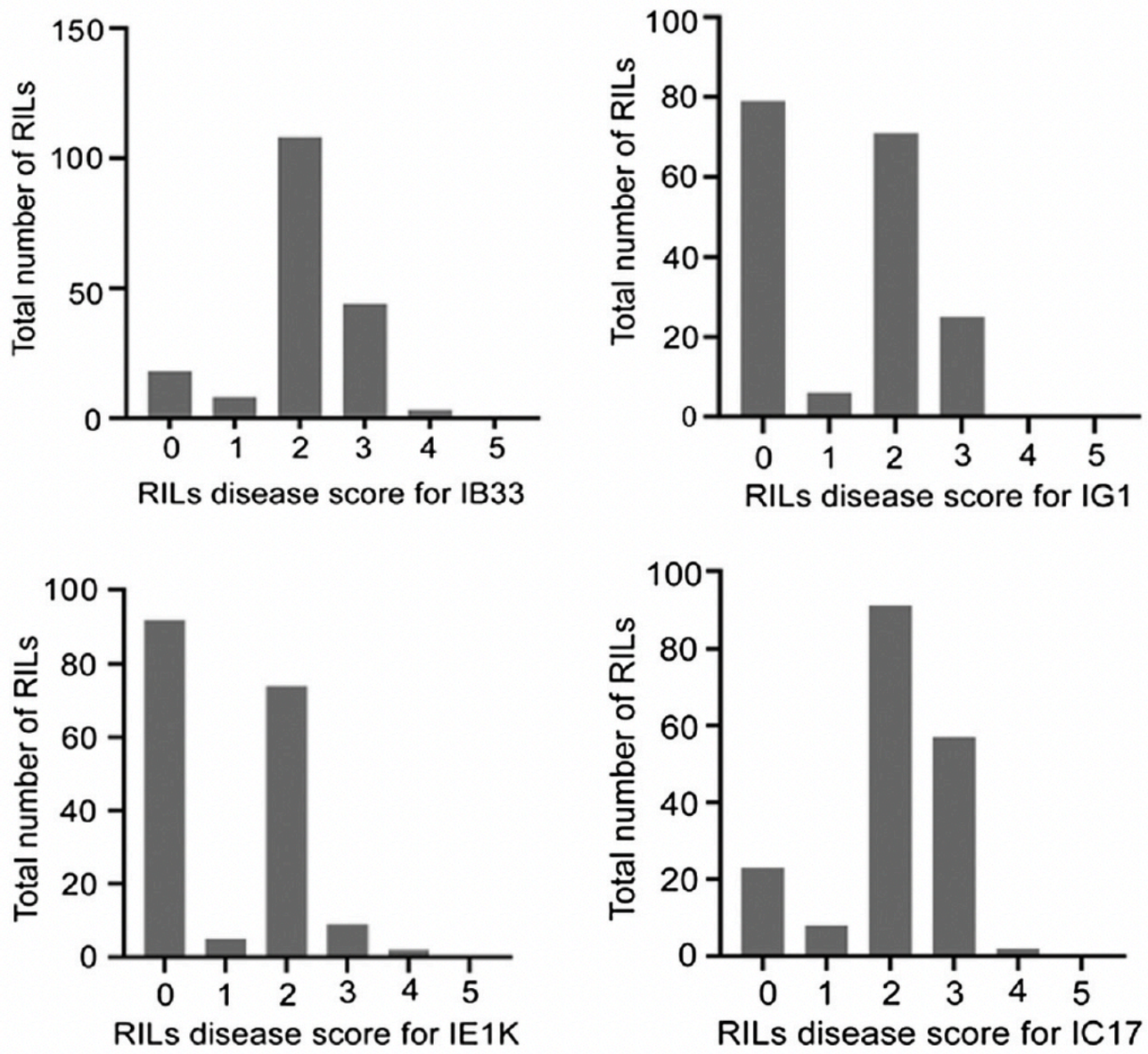

Fig. 2. Graphical representation of disease scores for recombinant inbred line (RIL) populations and parents using a rating scale of 0 to 5.

TABLE 2. A list of blast resistance quantitative trait loci (QTLs) identified, blast races, chromosomal location, and phenotypic variation for each QTL[a]

Blast isolate	Chr.	QTL	Left marker/right marker	LOD	PVE%	Additive value[b]	QTL source[c]
IB33	12	*qBLAST12*	S12_7748829/S12_15804848	9.167593	21.1152	0.05774	RR14
IC17	12	*qBLAST12*	S12_7748829/S12_15804848	7.791926	18.25706	0.06377	RR14
IG1	12	*qBLAST12-1*	S12_6229842/S12_15804848	5.090207	12.33895	0.08048	RR14

[a] LOD, limit of detection; PVE%, percent variance explained.
[b] A positive value shows that the source of the allele was RR14, whereas a negative value indicates aus-196.
[c] Sources of resistant genes RR14.

IG1 contained *Pi-ta* (144 bp) and *Ptr* (241 bp) detected in parental line RR14 (Supplementary Table S4), suggesting that *qBLAST12* identified in the present study is indistinguishable from *Ptr* and *Pita* present in RR20. Further fine mapping will be required for conclusive determination.

Discussion

Rice production is threatened by blast disease worldwide. Development of rice varieties from novel sources that confer broad-spectrum and durable resistance to rice blast disease is therefore a

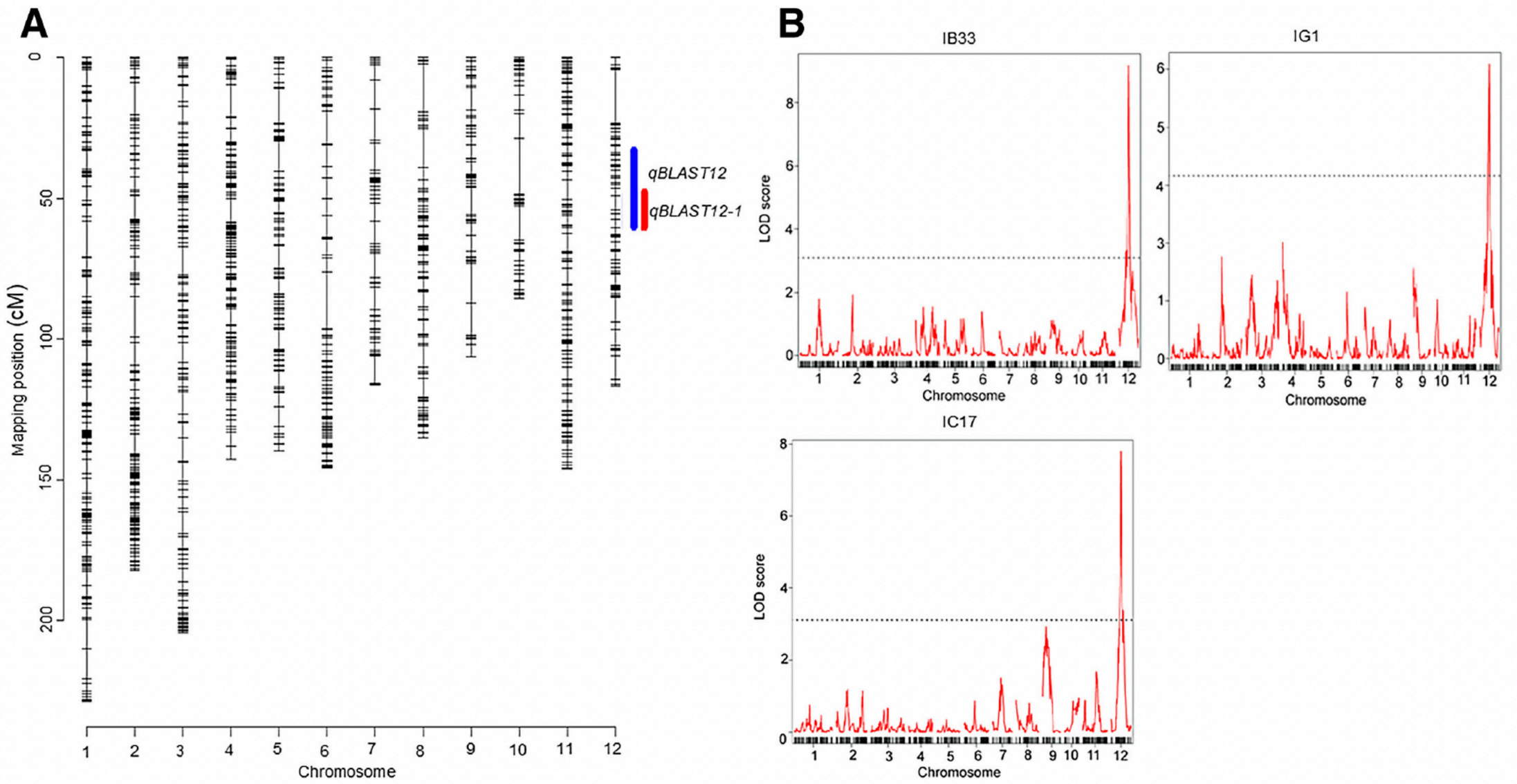

Fig. 3. A, High-resolution genetic map with mapped blast resistance quantitative trait loci (QTLs). Major QTLs were detected using blast races IB33, IC17, and IG1. **B,** Manhattan plots showing the associations between individual single-nucleotide polymorphisms and resistance to *Magnaporthe oryzae* isolates IB33, IG1, and IC17. LOD, logarithm of the odds.

TABLE 3. A list of blast resistance genes co-located with the quantitative trait loci identified in this study

Genomic loci or quantitative trait loci	Number of putative genes	Number of candidate genes	Disease-resistant genes	Disease resistance domain present[a]
qBLAST12	869	10	*LOC_Os12g17090*	NB-ARC/LRR
			LOC_Os12g17340	NB-ARC/LRR
			LOC_Os12g17410	NBS-LRR/NB-ARC
			LOC_Os12g17420	NB-ARC
			LOC_Os12g17430	ARM
			LOC_Os12g17490	NB-ARC
			LOC_Os12g18360	
			LOC_Os12g18374	
			LOC_Os12g18729	
			LOC_Os12g25170	
qBLAST12-1	1,308	19	*LOC_Os12g11680*	RLP23-LRR
			LOC_Os12g11720	
			LOC_Os12g11860	
			LOC_Os12g11930	
			LOC_Os12g11940	NB-ARC
			LOC_Os12g12010	RX-LIKE-CC/NB-ARC
			LOC_Os12g12120	NB-ARC/LRR
			LOC_Os12g13550	NB-ARC/LRR
			LOC_Os12g14330	NBS-LRR/NB-ARC
			LOC_Os12g17090	NB-ARC
			LOC_Os12g17340	ARM
			LOC_Os12g17410	NB-ARC
			LOC_Os12g17420	
			LOC_Os12g17430	
			LOC_Os12g17490	
			LOC_Os12g18360	
			LOC_Os12g18374	
			LOC_Os12g18729	
			LOC_Os12g25170	

[a] LRR, NB-ARC, NBS-LRR, RX-LIKE-CC, and ARM are disease resistance protein domains.

major goal for rice breeders. Weedy rice, although an undesirable competitor of cultivated rice, is known to possess novel genes resistant to blast (Lee et al. 2011; Zhao et al. 2022). In the present study, we report the identification of two major blast resistance QTLs using a mapping population derived from a cross between a U.S. weedy rice strain and an *aus* rice variety. Both loci mapped to the middle of chromosome 12; *qBLAST12* controls variation in resistance to blast races IB33 and IC17, and *qBLAST12-1*, which overlaps and extends beyond *qBLAST12*, controls variation in resistance to blast race IG1. For both loci, resistance is conferred by the allele present in the weedy rice parent. In a previous study, a major QTL for resistance to blast race IB33 was identified in another U.S. BHA weedy rice accession, and it mapped to the same general region of chromosome 12 (Liu et al. 2015). Race IB33 is highly virulent and is capable of overcoming the resistance of the major *R* gene *Pi-ta/Pi39(t)/Ptr*, which has been widely deployed in cultivars.

The common practice for mapping major-effect QTLs is to use one resistant and one susceptible parent to generate a mapping population that allows for distinct segregations to capture the maximum number of QTLs. In the present study, initially, five predominant U.S. *M. oryzae* races (IB49, IB33, IC17, IE1K, and IG1) were used for the mapping population and parents. Except IB49, all exhibited distinct phenotypes in segregating progeny, which allowed us to identify two overlapped resistance QTLs in a weedy rice genotype.

The identification of a major QTL in this study that contains *Pi-ta/Pi39(t)/Ptr* alleles using blast race IB33 supports the hypothesis that weedy rice in the United States has evolved a competitive ability to fight biotic stress. Some of these novel blast resistance QTLs present in weedy rice could have been introduced unintentionally from its domesticated ancestors (Li et al. 2004; Thurber et al. 2014). Analysis of the physical location of *qBLAST12* revealed the presence of several major *R* genes in this pericentromeric region of chromosome 12. One of these is *Ptr* (corresponding *LOC_Os12g18729*), which encodes an atypical blast resistance protein with armadillo repeats and was previously reported to confer broad-spectrum resistance in the U.S. rice cultivar Katy (Zhao et al. 2018). Another is *Pi-ta* (corresponding *LOC_Os12g18360*) (Bryan et al. 2000), which encodes a predicted NBS-LRR domain that directly interacts with the product of *M. oryzae* effector gene *AVR-Pita,* resulting in disease resistance (Jia et al. 2000). Furthermore, *Pi-39(t)* (*LOC_Os12g18374*) (Liu et al. 2007), located next to *Pita*, is yet another major blast *R* gene within *qBLAST12*. The two blast races against which *qBLAST12* shows resistance, IB33 without *AVR-Pita* and IC17 with *AVR-Pita*, suggest that resistance is independent of *AVR-Pita/Pi-ta* interaction. Zhao et al. (2022) showed that the *Ptr* gene in BHA weedy rice was associated with resistance to blast race IB33. It is possible that resistance shown by *qBLAST12* could be mediated by *Ptr*. The majority of RILs with the *Ptr* marker HJ17-13 for weedy rice RR14 showed resistance to IB33 and IE1K without *AVR-Pita*, IC17 with *AVR-Pita*, and IG1 with *AVR-Pita*, thus further pointing to the possibility of *Ptr* being *qBLAST12*, which is independent from *AVR-Pita*. However, we cannot rule out the possibility of the eight other *R* genes that were identified in this study within *qBLAST12* (Table 3), including *LOC_Os12g17410* (Yu et al. 2018) and *Pi-39(t)* (*LOC_Os12g18374*) (Liu et al. 2007) that were previously reported to be responsible for blast disease resistance in rice. The identification of the major QTLs, *qBLAST12* and *qBLAST12-1*, in this study, which confer resistance to blast races IB33, IC17, IG1, further supports the crucial role of rice chromosome 12 in offering resistance against blast disease.

Partial resistance to rice blast fugus is manifested by the presence of fewer and smaller lesions on the leaf blade. Blast-resistant rice varieties possessing a single *R* gene confer nondurable resistance, as they lose their resistance with time (Babujee and Gnanamanickam 2000). Varieties with durable resistance may harbor more than one *R* gene (Zhu et al. 2012). Previous studies showed that pyramiding of blast *R* genes into rice varieties is an effective approach to develop high and durable resistance (Hittalmani et al. 2000; Jiang et al. 2012). Although the majority of RILs in our mapping population showed the presence of fewer and smaller lesions on infected plant leaves, with scores of 1 and 2 (Table 2), we were unable to detect minor-effect QTLs. However, within *qBLAST12* and *qBLAST12-1* (Table 3), in addition to major known *R* genes *Ptr*, *Pita*, and *Pi-39(t)*, several other putative disease *R* genes were found, and they can be deployed for gene pyramiding to develop broad-spectrum and durable rice varieties.

Conclusions

Previously, we showed that the *Pi-ta/Pi39(t)/Ptr* locus in rice variety Katy is responsible for race-specific resistance to blast disease and a *Ptr* allele in a weedy genotype is responsible for preventing infections of a virulent laboratory blast race. In the present study, using inexpensive reproducible phenotypic data under greenhouse conditions, we mapped two major QTLs, *qBLAST12* and *qBLAST12-1*, at the *Pi-ta/Pi39(t)/Ptr* locus using RILs derived from a biparental cross of another distinct U.S. weedy rice genotype and an *aus* rice variety (aus-196). Resistance alleles for both QTLs were from the weedy rice parent, demonstrating the evolutionary advantages of weedy rice and the importance of weedy rice as a novel source of blast *R* genes. These findings add to new genetic markers and resources that will be beneficial to rice breeders through a marker-assisted breeding approach to introgress *R* genes into susceptible rice varieties.

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