

An Allelic Variant of the Broad-Spectrum Blast Resistance Gene *Ptr* in Weedy Rice Is Associated with Resistance to the Most Virulent Blast Race IB-33

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Abstract

Rice resistance (*R*) genes have been effectively deployed to prevent blast disease caused by the fungal pathogen *Magnaporthe oryzae*, one of the most serious threats for stable rice production worldwide. Weedy rice competing with cultivated rice may carry novel or lost *R* genes. The quantitative trait locus *qBR12.3b* was previously mapped between two single nucleotide polymorphism markers at the 10,633,942-bp and 10,820,033-bp genomic positions in a black-hull-awned (BHA) weed strain using a weed-crop-mapping population under greenhouse conditions. In this study, we found a portion of the known resistance gene *Ptr* encoding a protein with four armadillo repeats and confers a broad spectrum of blast resistance. We then analyzed the sequences of the *Ptr* gene from weedy rice, *Ptr*^{BHA}, and identified a unique amino acid glutamine at protein position 874. Minor changes of protein conformation of the *Ptr*^{BHA} gene were predicted through structural analysis of *Ptr*^{BHA},

suggesting that the product of *Ptr*^{BHA} is involved in disease resistance. A gene-specific codominant marker HJ17-13 from *Ptr*^{BHA} was then developed to distinguish alleles in weeds and crops. The *Ptr*^{BHA} gene existed in 207 individuals of the same mapping population, where *qBR12.3b* was mapped using this gene-specific marker. Disease reactions of 207 individuals and their parents to IB-33 were evaluated. The resistant individuals had *Ptr*^{BHA} whereas the susceptible individuals did not, suggesting that HJ17-13 is reliable to predict *qBR12.3b*. Taken together, this newly developed marker, and weedy rice genotypes carrying *qBR12.3b*, are useful for blast improvement using marker assisted selection.

Keywords: disease resistance, *Magnaporthe oryzae*, *Ptr* allele, weedy rice

Rice blast disease, caused by the filamentous fungus *Magnaporthe oryzae* (T.T. Hebert) M.E. Barr (synonym: *Pyricularia oryzae*), is one of the most damaging rice diseases worldwide (Pennisi 2010). Blast disease is primarily managed by the use of resistance (*R*) genes and fungicides. An estimated \$68 million is allocated to prevent rice blast disease annually even though <2% of world rice is grown in the United States. Hence, a continued search for new sources of *R* genes is necessary to secure stable rice production. In the United States, major *R* genes, such as *Pi-ta*/*Ptr*, *Pi-b*, *Pi-z*, and *Pik*, have been effectively deployed providing overlapped resistance to 11 common races of *M. oryzae*, namely, IA-45, IB-1, IB-45, IB-49, IB-54, IC-17, ID-1, IE-1, IE-1k, IG-1, and IH-1 (Jia et al. 2009, 2019).

Previously, we showed that the *Pi-ta* and *Ptr* genes in ‘Katy’ are effective blast *R* genes against a wide range of *M. oryzae* races and the *Ptr* gene is located 210 kb from *Pi-ta* (Zhao et al. 2018). *Ptr* confers resistance to a wide range of blast races together with *Pi-ta* and encodes two predicted protein isomers (905 and 864 amino acids) with four armadillo repeats (Zhao et al. 2018). Broad spectrum resistance of *Ptr* was highly similar to that of *Pi-ta2* (Meng et al. 2020). The *Ptr* gene encoding a protein with armadillo repeats without the U box presumably acts as a pathogen receptor similar to that

of the nuclear binding site and leucine-rich repeat receptors. Exactly how *Ptr* detects pathogen-signaling molecules in triggering a defense response is unknown.

A historical survey demonstrated that the U.S. *M. oryzae* population has become more diverse and virulent over time (Wang et al. 2017b). Race IB-33, a laboratory-derived strain, is thus far one of the most virulent races in the United States (Lee et al. 2009). Several virulent races overcoming *Pi-ta* were discovered in the rice cultivar Banks (Zhou et al. 2007). As an alternative to major *R* genes, minor *R* genes referred to as quantitative trait loci (QTL) are more durable and not race-specific. Weedy species of rice competing with cultivated rice have evolved novel blast resistance QTL (Delouche et al. 2007; Jia and Gealy 2018; Liu et al. 2015). Among the identified blast resistance QTL, *qBR12.3b* identified by IB-33 in one black-hull-awned (BHA) weed strain has a portion of the *Ptr* gene (Liu et al. 2015; Zhao et al. 2018). It is unknown if the *Ptr*^{BHA} allele is associated with blast resistance.

The objectives of this study were to examine the *Ptr* allele in a BHA-crop-mapping population; identify the critical amino acid residues necessary for *Ptr*^{BHA}-mediated plant innate immunity; and develop the *Ptr*^{BHA}-specific DNA marker from a portion of the gene to improve rice blast resistance via marker-assisted selection (MAS).

Materials and Methods

Plant materials. A recombinant inbred line (RIL) population consisting of 207 lines derived from a cross between a BHA type PI 653419 (MS-1996-9) and the *indica* variety Dee Geo Woo Gen (DGWG) was used in this study. These RIL lines were created by a single seed descent method in the greenhouse and field (Jia et al. 2022).

Pathogen isolate, culture, and spore suspension. *M. oryzae* race (isolate) IB-33(FL9) was generously provided by Dr. Fleet Lee, and IC-1(BRFD-2F-2), IC-17(ZN57), ID-1(ZN42), IE-1(ZN13), and IE-1k(TM2) were used for analysis (Lee et al. 2009; Liu et al. 2015). Single spores were purified using a procedure described by Jia (2009). Spores were stored on desiccated filter papers at -20°C. Desiccated filter paper pieces with mycelia and spores were transferred from the

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–20°C freezer to oatmeal medium plates (BD Difco, Franklin, NJ) for 7 days at 22°C under dark or white fluorescent light in the fungal incubator (Percival, Perry, IA). Spores were collected by sterilizing 0.25% gelatin solution and filtered using four layers of cheesecloth. The spore concentrations in the suspensions were determined using a hemacytometer under a microscope and adjusted to 1×10^5 spores/ml using 0.25% gelatin solution for inoculation.

Greenhouse inoculation and evaluation. Germinated rice seeds were planted in a black plastic cell ($2.9 \times 4.0 \times 5.6$ cm) with a hole at the bottom. A total of 96 cells with seeds and sterilized local silt loam soil from the field were randomly placed in a tray (25.4×50.8 cm). For each line, four to seven seedlings were grown in a greenhouse at 24 to 30°C under an 8-h dark and 16-h light cycle until the V3 to V4 leaf stages (approximately 18 days after sowing). Each tray was inoculated with 25 ml of filtered spore suspension using an artist's airbrush in a black plastic bag, and incubated for 24 h at 21 to 24°C. Then, inoculated plants were transferred to a cooling room with an 8-h light and 16-h dark cycle with 80%

relative humidity at 24°C for an additional 6 days. Disease evaluation was determined using a categorical rating system modified from Liu et al. (2015) as follows: 0 to 2 = resistant and 3 to 5 = susceptible, where 0 = no visible lesion, 1 = a few small point lesions, 2 = lesion diameter size <2 mm without visible fungal mycelia, 3 = 10% of leaf area with >2-mm diameter lesions with visible fungal mycelia, 4 = >10% but <50% of the leaf with >3-mm diameter lesions, and 5 = >50% of leaf area with >3-mm diameter lesions (Supplementary Fig. S1). One single disease reaction score was given to four to seven seedlings per cell and disease reactions were repeated three times and its mean value was used for analysis. A dissecting Nikon microscope (Tokyo, Japan) was used to verify the absence of fungal mycelia for rating at “2.”

DNA sequence analysis. *qBR12.3b* was previously mapped between two single nucleotide polymorphism (SNP) markers at the 10,633,942-bp and 10,820,033-bp genomic positions (Liu et al. 2015) excluding *Pi-ta* (LOC_Os12g18360; Bryan et al. 2000) and another nuclear binding site and leucine-rich repeat protein *Pi39(t)/Pi42(t)* LOC_Os12g18374 (Kumar et al. 2010; Liu et al. 2007), based on the Nipponbare reference sequence in the program MSU v7.0 (<http://rice.uga.edu/>; Supplementary Table S1). The *Ptr* genomic sequence as well as 2 kb upstream and downstream of BHA, straw-hull-awnless (SH), and DGWG (Li et al. 2017), and the *Ptr* protein coding DNA sequences of BHA and ‘Katy’ were retrieved from Zhao et al. (2018). Multiple sequence alignments were performed using the tool ClustalX2 (<https://evomics.org/resources/software/bioinformatics-software/clustal-x/>; Larkin et al. 2007) and BioEdit (<https://bioedit.software.informer.com/>; Hall 1999).

Protein prediction and analysis. The protein secondary structure and disorder prediction were analyzed in the program PSIPRED (<http://bioinf.cs.ucl.ac.uk/psipred/>; Buchan and Jones 2019), and the three-dimensional (3D) protein structure was predicted by the program Phyre2 (Kelley et al. 2015) with normal modeling mode and the program RaptorX (<http://raptorx.uchicago.edu/>; Wang et al. 2017a) with default parameters. The software EzMol (<http://www.sbg.bio.ic.ac.uk/ezmol/>) was used to visualize and analyze the protein structure (Reynolds et al. 2018).

DNA marker development and PCR amplification. Two pairs of primers, HJ17-13 (forward 5'-CACCCTAATTTGTACTTTCTC CG-3' and reverse 5'-TCGGTTTGCATTACTCCCTC-3'), and HJ17-11 (forward 5'-TGGGTATTGGCGATGATTC-3' and reverse 5'-TGTGGCCCTAACACTTGGGA-3') were designed according to the identified putative DNA variations between BHA and DGWG using the software Primer Premier 5 (<http://www.premierbiosoft.com/primerdesign/>; Bryan et al. 2000; Zhao et al. 2018). PCRs were performed in a 50- μ l volume with 100 ng of genomic DNA, 25 μ l of 2 $\times$ Taq PCR Master Mix (Cat. No. 201445; QIAGEN, Hilden, Germany), and 0.2 μ M of each primer. A touchdown program was used to increase the specificity of PCR amplification as follows: 94°C for 3 min; five cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min; five cycles of 94°C for 30 s, 57.5°C for 30 s, 72°C for 1 min; 25 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min; and a final extension of 72°C for 10 min. PCR products were separated by 1% Tris-acetate-EDTA agarose gel electrophoresis and stained with SYBR

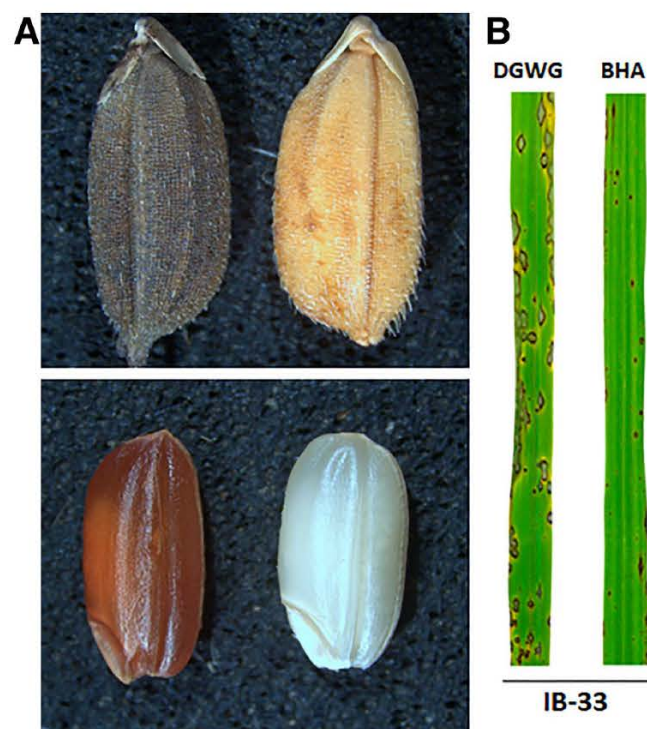


Fig. 1. Parents black-hull-awned (BHA) and Dee Geo Woo Gen (DGWG) and their disease reaction. **A**, Seeds of indicated rice variety: Upper left, black, BHA and upper right, tan, black-hull-awnless; lower left, brown, DGWG with hull; lower right, white, DGWG without hull. **B**, Resistant reaction of weedy rice BHA and DGWG. BHA has resistant allele *Ptr*^{BHA} and is resistant to the race IB-33. DGWG is susceptible. Photograph was taken 7 days after seedlings were inoculated with IB-33.

Table 1. Analysis of reactions of recombinant inbred lines to *Magnaporthe oryzae* races and existence of *R* genes

Race	Disease reaction		<i>P</i> value	Number of line disagrees with the <i>Ptr</i> marker	Conclusion	
	Number of resistant lines	Number of susceptible lines			Ratio for χ^2 test ^a	Association of disease reaction with the <i>Ptr</i> marker ^b
IC-1	66	141	0.00	48	Not 1R:1S	<i>R</i> gene cannot be determined
IC-17	52	155	0.00	51	Not 1R:1S	<i>R</i> gene cannot be determined
ID-1	67	140	0.00	51	Not 1R:1S	<i>R</i> gene cannot be determined
IE-1	114	89	0.08	46	1R:1S	<i>R</i> gene cannot be determined
IE-1K	79	128	0.03	39	Not 1R:1S	<i>R</i> gene cannot be determined
IB-33	95	112	0.24	3	1R:1S	<i>Ptr</i> ^{BHA}

^a Expected resistance lines, *R* = 103.5; susceptible lines, *S* = 103.5. Not = The ratio of resistant individual with susceptible individual is not 1:1.

^b Association with the *Ptr* allele with respected disease reaction cannot be determined because of the large number of disagreements of marker and phenotype.

Safe cyanine dye (Cat. No. S33102; Invitrogen, Waltham, MA). The target fragment was cut under an ultraviolet light and purified using a QIAquick Gel Extraction Kit (Cat. No. 28704; QIAGEN) according to the manufacturer's instructions. The PCR amplicon was verified by sequence analysis on an ABI 3730XL DNA Analyzer (Applied Biosystems/Thermo Fisher Scientific, Waltham, MA) with 0.5 µl of BigDye v3.1 reactant (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems/Thermo Fisher Scientific) at the U.S. Department of Agriculture's Agricultural Research Service Mid South Area Genomics and Bioinformatics Research Unit (Stoneville, MS).

Genotyping. Genomic DNA from rice seedling leaves was extracted using a QIAamp DNA Mini Kit (Cat. No. 51306; QIAGEN) according to the manufacturer's protocol. DNA concentrations were determined with a Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Forward primers of identified insertion/deletion (InDel) markers were labeled with 5' HEX fluorescent dye (Integrated DNA Technologies, Coralville, IA). For genotyping, PCR analyses were run in a total volume of 20 µl, including 25 ng of genomic DNA, 10 µl of 2 × Taq PCR Master Mix (Cat. No. 201445; QIAGEN), and 0.2 µM of each primer. The following PCR routine was used: 94°C for 3 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension of 72°C for 10 min. PCR products were denatured at 94°C for 5 min before running on the ABI 3730 DNA Analyzer (Applied Biosystems). The allele size was determined with the software GeneMapper v3.7 (https://genemapper.software.informer.com/3.7/; Applied Biosystems).

Results

Reactions of RILs to *M. oryzae* races. The reactions of the weedy rice genotypes to *M. oryzae* were different from that of the cultivated rice varieties, as they often exhibited a more hypersensitive cell death. The reaction of cultivated rice DGWG ranged from 3 to 5, whereas that of weedy rice BHA ranged from 1 to 2 depending on the concentration of conidia in the inoculant, seedling growth stage/health, and incubation conditions. A typical reaction of a BHA genotype to the race IB-33 of *M. oryzae* is shown in Figure 1. The reactions of an RIL population derived from the cross of DGWG with BHA were genetically analyzed (Table 1). Based on the χ^2 test, a single dominant nuclear *R* gene was associated with the resistance to races IE-1 and IB-33 (Table 1).

Sequence analysis of the *Ptr* alleles. Previously, the resistance QTL *qBR12.3* was identified with the race IB-33 from a weed crop-mapping population (Liu et al. 2015). The QTL *qBR12.3* flanked by SNP markers S12_10633942 and S12_10820033 spans a 186,137-bp region that contain 30 annotated genes including an SNP in the first

exon of the *Ptr* gene at the border S12_10820033 (Fig. 2A; Supplementary Table S1). Because the *Ptr* gene is the only known *R* gene in this area, we investigated whether the *Ptr* allele in weedy rice is associated with resistance at *qBR12.3*. To examine whether the *Ptr* allele in BHA is associated with blast resistance, the DNA variations in the *Ptr* gene of weedy rice, 2,718 bp of the *Ptr* protein coding region in 'Katy', and 2,730 bp in BHA, SH, 'Katy', and DGWG, were analyzed. We found two synonymous substitutions, 11 nonsynonymous substitutions, and one 12-bp InDel between them, and most of the DNA variations were identified in the fourth exon (Fig. 2B). Of these, the *Ptr* haplotype in the resistant weedy rice BHA was highly similar to *Ptr* in susceptible rice DGWG except for two nonsynonymous substitutions at the 2,609- and 2,611-bp positions. However, the nonsynonymous substitution located at 2,611 bp was identical between the susceptible 'Katy' and the resistant BHA, indicating that a nonsynonymous substitution at nucleotide position 2,609 results in an amino acid change to Glutamine from Proline. This change potentially plays a critical role in BHA's blast resistance to IB-33.

To determine if the above-mentioned amino acid differences of the product of the *Ptr* alleles can alter the protein structure, we first analyzed the *Ptr* protein secondary structures *in silico*. The program Phyre 2 (<http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index>) compares the *Ptr* protein with a known structure of similar protein template (c3l6yA_) that identified 330 amino acid residues with 98.7% confidence and revealed a few differences in 3D structures (Fig. 3). Three-dimensional structures of 289 to 783 amino acids of the protein positions were shown, and differences were indicated in red arrows (Fig. 3). We then used the program RaptorX to predict the structure by deep learning. Obvious structural differences of the *Ptr*

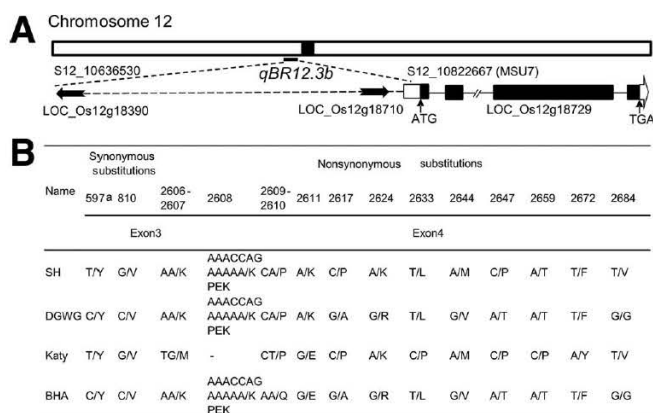


Fig. 2. Genome organization of the *Ptr* gene and a gene-specific marker for *Ptr*. **A**, The *Ptr* gene was mapped at the end of *qBR12.3b*. **B**, Polymorphic regions of the nucleotides and amino acids of open reading frame of the *Ptr* gene in rice susceptible genotypes, straw-hull awnless (SH), Dee Geo Woo Gen (DGWG), 'Katy', and resistant genotype black-hull-awned (BHA). The longer isomer, consisting of 905 amino acids of the *Ptr* protein and the genome location from 'Katy', were used. Single-letter abbreviations were used for each amino acid.

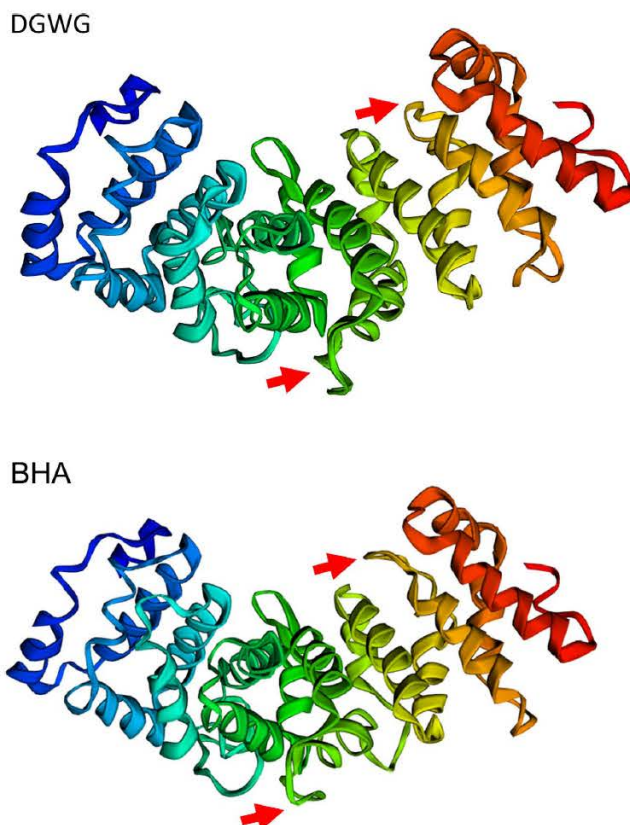


Fig. 3. Three-dimensional (3D) structure prediction of the *Ptr* protein in Dee Geo Woo Gen (DGWG) and black-hull-awned (BHA) biotypes. The 3D model of the *Ptr* protein was predicted with the Phyre 2 server program (<http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index>) in normal mode and shown using the program EzMol (<http://www.sbg.bio.ic.ac.uk/~ezmol/>). The 3D high-resolution protein structure template of c3l6yA_ was used as template in the comparative modeling procedure. The difference between DGWG and BHA is indicated with the red arrows.

proteins were also detected between BHA and DGWG (Fig. 4). Notably, $\text{Ptr}^{\text{BHA}874\text{Q}}$ is in a region of the protein that is more disordered with less defined strand, helix, and coil structures, and with a predicted stronger binding function than $\text{Ptr}^{\text{DGWG}874\text{P}}$. The results suggest that these altered structures may impact the detection of pathogen signaling molecules to trigger *Ptr*-mediated plant immunity.

Fine mapping and development of a gene-specific marker for the *Ptr* allele in BHA. To validate the results of Liu et al. (2015), we analyzed the 2 kb upstream and downstream of genomic sequences of the *Pi-ta* and *Ptr* genes, identifying a 1-bp and 6-bp InDel in the 3' untranslated region (UTR) of *Pi-ta* and *Ptr* between BHA and DGWG, respectively (Bryan et al. 2000; Jia et al. 2000; Fig. 5A; Supplementary Fig. S2). Two flanking markers were identified. HJ17-11 from the 3' UTR of the *Pi-ta* produced a 144-bp amplicon from BHA and a 143-bp amplicon from DGWG, and HJ17-13 from the 3' UTR of the *Ptr* gene produced a 241-bp amplicon from BHA and a 247-bp amplicon from DGWG (Fig. 5B).

Trait and marker relationship. To determine if the 6-bp InDel cosegregates with resistance, a total of 207 RILs derived from the BHA and DGWG cross were selected for further analysis. Using the *Ptr* gene-specific InDel marker HJ17-13, a 241-bp fragment was amplified from 98 RILs with disease scores of 0 to 2, while a large 247-bp fragment was obtained from other 109 RILs that exhibited disease scores 3 to 5. Three RIL lines, 35, 231, and 261, carrying the *Ptr* 241-bp fragment had inconsistent reactions between scores 2 and 3 depending on plant and environmental conditions after inoculations (Supplementary Table S2; Supplementary Fig. S1). We also found that marker HJ17-11 provides the same genotyping information with that of HJ17-13, suggesting that there is no recombination

between these two markers, and both can be used to predict blast resistance in BHA (Supplementary Table S2).

The reaction of RILs to the race IE-1 is also associated with a single locus; however, 46 RILs whose reactions disagreed with the existence of the *Ptr* allele indicated that the *Ptr* marker is not efficient to predict the resistance effect to the race IE-1. Together, these findings suggest that the InDel marker HJ17-13 identifies a gene associated with resistance to the race IB-33 in BHA and its progeny with 98.6% accuracy and to the race IE-1 with 77.8% accuracy (Table 1; Supplementary Table S2).

Discussion

Previously, we showed that the *Pi-ta* and *Ptr* genes in 'Katy' are effective blast *R* genes to a wide range of blast races including IC-1, IC-17, ID-1, and IE-1, except for IE-1k and IB-33 (Zhao et al. 2018). In this study, we found that the Ptr^{BHA} allele from a weedy rice genotype is effective for different *M. oryzae* races, including virulent races IB-33 and IE-1. Weedy rice is believed to be unintentionally introduced and may have *R* genes absent in cultivated rice (Gross et al. 2010; Jia and Gealy 2018; Thurber et al. 2013, 2014). Alternatively, weedy rice can be a byproduct of de-domestication and may create new *R* genes (Chen et al. 2004; Jia and Gealy 2018, Lee et al. 2011; Thurber et al. 2013, 2014). In the United States, BHA and SH are the two genetically distinct primary ecotypes of weedy rice with novel blast *R* genes (Lee et al. 2011; Liu et al. 2015; Reagon et al. 2010; Thurber et al. 2010). In this study, the *Pi-ta* and *Ptr* genes in the domesticated rice variety 'Katy' does not confer resistance to IB-33, while the allele of *Ptr* in BHA confers resistance to

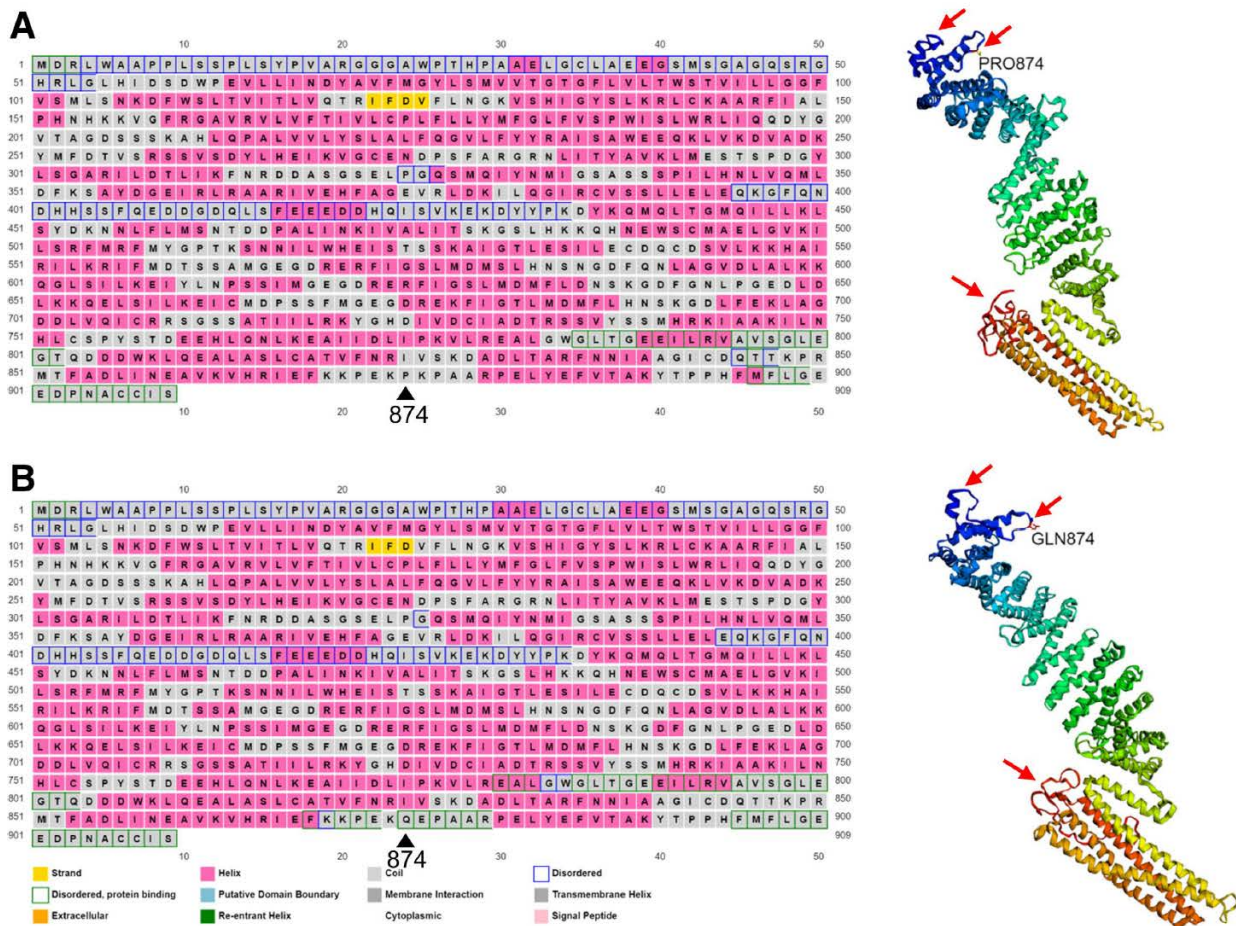


Fig. 4. Predicted structure of the *Ptr* proteins in rice. Minor structure alterations are indicated by black arrows. **A**, Two- and three-dimensional (3D) structures of Ptr^{DGWG} (909 amino acids) in Dee Geo Woo Gen (DGWG). **B**, Ptr^{BHA} (909 amino acids) in black-hull-awned biotypes (BHA). The structure of the *Ptr* protein of the longer isomer was based on the RaptorX server program (<http://raptorx.uchicago.edu/ContactMap/>). Note: The 3D protein structure is different using the Phyre2 and RaptorX server programs. Phyre2 models (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) only cover 289 to 783 amino acids of the *Ptr* protein while RaptorX covers the entire protein (909 amino acids). Both analyses displayed structural difference between Ptr^{BHA} and Ptr^{DGWG} .

this race. The genome sequence analysis revealed that BHA rather than SH from the United States was closely related to the most ancient, cultivated rice (Li et al. 2017) and suggested that BHA had inherited an untapped recognition specificity. It is also possible that the novel resistance of BHA was generated by de-domestication processes such as gene flow or recombination followed by natural selection. In this regard, it is not surprising that BHA and SH have different blast *R* genes (Lee et al. 2011; Liu et al. 2015). Nevertheless, our findings indicate that weedy rice can be used as a novel source for blast resistance.

It is commonly believed that weedy rice is of *indica* origin. However, disease reactions of weedy rice are typically different from that of *indica* rice. Disease reaction of BHA to *M. oryzae* differed from that of *indica* varieties such as DGWG. Often, BHA has more brown spots presumably because of the rapid occurrence of hypersensitive cell death, an outcome of a disease-resistant reaction. We observed that BHA and the three progenies of BHA with *Ptr*^{BHA} had varied reaction scores 2 to 3 under 80% relative humidity after initial incubation in a sealed plastic bag (Supplementary Table S2). Variability in rice blast reactions was reported in Ou (1980). One possibility is that the defense response in BHA and these progenies were influenced by the humidity or other environmental conditions attributable to other genes segregating in these progenies. It is equally possible that resistance specificity of *Ptr*^{BHA} to the race IB-33 may also be involved in other modified genes in BHA that are regulated by environmental factors. A further detailed study of genetic makeups of these RILs and BHA is needed to substantiate these unusual disease responses and gain insights of variability of host immunity.

Analysis of the IRRI 3K-sequenced rice germplasm revealed that the *Ptr* allele from *indica* lacks 12 bp, resulting in alteration of four amino acids that are responsible for resistance to *M. oryzae* (Zhao et al. 2018). However, the *Ptr*^{BHA} allele has these 12 bp. In comparison with that of 'Katy', which is susceptible to IB-33, glutamine at position 874 of the *Ptr*^{BHA} protein versus proline was identified, indicating that glutamine plays an important role in detecting the IB-33 pathogen. First, glutamine is classified as a polar amino acid while

proline is a nonpolar amino acid, which may affect the protein surface and folding. Second, *Ptr*^{BHA874Q} is predicted as a disordered region potentially involved with protein binding (Figs. 3 and 4). Many disordered proteins can bind a structured partner and function via a disorder-to-order transition including the precise control of binding specificity without impacting affinity (Mészáros et al. 2009). Finally, *Ptr*^{BHAQ874P} may change the 3D protein structure and impact *Ptr* protein activity (Figs. 3 and 4). For example, a single amino acid determining resistance specificity was observed in blast *R* genes, *Pi-ta* (Bryan et al. 2000). In the *Pi-ta* protein, alanine at position 918 determines resistance specificity and *Ptr* was predicted to be a helper, a failsafe for *Pi-ta* (Zhao et al. 2018), demonstrating that plants have evolved a simple mechanism to cope with biotic stress. Together, these findings are useful for machine learning to predict critical amino acid residues of *R* proteins. Further examination of interaction specificity of the *Ptr* and *Pi-ta* proteins with the pathogen effector and/or interacting proteins will help understand mechanisms of pathogen recognition in triggering effective defense response. The resulting knowledge is useful for developing effective crop protection methods that are environmentally benign.

For a short-term benefit, InDel markers can be highly useful for MAS for improving blast resistance. MAS has been routinely used for incorporating new *R* genes in breeding lines worldwide. HJ17-13 derived from a portion of *Ptr*^{BHA}, with its high correlation with resistance to IB-33, can be used to examine the accuracy of predication for blast resistance in collections of weedy rice and rice germplasm. If confirmed, HJ17-13 can be used to monitor blast resistance from a wide range of resistant donors for rice breeding programs.

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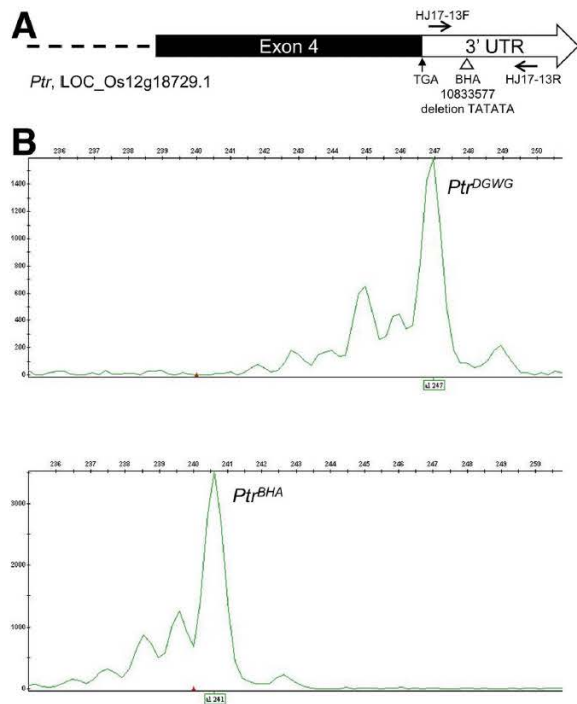


Fig. 5. Graphic presentation of the genomic regions of the *Ptr* gene. **A**, Schematic presentation of the location of *qBR12.3b* and genome organization of the *Ptr* locus. UTR, untranslated region. **B**, Graphics showing predicted sizes of the *Ptr* allele in the Dee Geo Woo Gen (DGWG) and black-hull-awned (BHA) biotypes. The y axis indicates relative fluorescent units; the x axis indicates base-pair size.

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