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Detection of blast resistance genes in inbred rice lines using site-specific blast races

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Abstract Blast disease attacks by *Pricularia oryzae* on lowland rice were quite extensive in the Province of Bengkulu, Indonesia. Screening of 19 lines inoculated with four local races (333-BT, 001-BU, 043-RM, and 373-BS) revealed variations in blast disease resistance. There were nine rice lines (G7, G8, G9, G11, G13, G14, G15, G18, and G19) that showed resistance to blast disease with lower severity. There are found the race-sensitive lines with high virulence (333-BT and 373-BS), namely G3, G4, G6, G12, and G17. In addition, races 333-BT and 373-BS tended to show high virulence in sensitive varieties of Kencana Bali. Almost all lines had low levels of severity in low-virulence races (001-BU and 043 RM). Detection of blast-resistant genes using specific primers is found to be detected the existence of the genes Pib, Pii, Pi5, and Pita2. This study found that the G7, G8, G9, G11, G13, G14, G15, G18, and G19 lines are shown to be the potential for further evaluation because they contain the Pii, Pi5, and Pita2 genes, which have multigenic and broad-spectrum blast resistance.

Keywords: Bengkulu isolates, Blast resistance, Broad spectrum, Race, Virulence

Introduction

Rice is widely grown in nine districts across the Province of Bengkulu, Indonesia, and is cultivated for lowland rice (irrigated) and upland rice (rainfed) production. Previous field monitoring indicated that blast-disease attacks on lowland rice caused by the fungus *Pyricularia oryzae* have been widely reported in the study districts, although the magnitudes of these attacks have not been well documented. Nevertheless, Sudir *et al.* (2014) reported that blast diseases of upland rice can infect lowland rice varieties, including IR64. High diversity and changes in this fungus race, as well as its virulence, contribute to the reduction of rice variety resistance (Mulyaningsih *et al*., 2016; Liu *et al*., 2017; Sheoran *et al*., 2021). Another factor that may cause changes in variety resistance is the in

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the particular area. Planting rice varieties with selected genes for resistance different race composition of *P. oryzae* found in specific areas. In specific areas or locations, the choice of rice varieties with resistance genes to Pyricularia should match the race composition (Sudir *et al.,* 2014). In order to successfully plant resistance varieties of the chosen gene, it's necessary to have data on the race composition of *Pyricularia* in a specific region. Thus, monitoring *Pyricularia* races in rice agro-ecosystems is urgently needed, especially in areas endemic to blast disease (Xu *et al*., 2021). Planting rice varieties with selected genes for resistance must be adjusted to the race composition of *Pyricularia* in a specific area or location (Sudir *et al*., 2014). In a specific region, it's essential to confirm the race composition of *Pyricularia* before planting the resistance gene variety. Monitoring *Pyricularia* races is essential in all rice agro-ecosystems, especially in areas prone to blast disease (Xu *et al*., 2021).

Research conducted by Sudir *et al*. (2014) to monitor blast races in six districts of Central Java (Indonesia) during the growing season of 2013 found 122 isolates of *P. grisea* and 23 races. Among them, races 013 and 153 were the most dominant, with respective magnitudes of 9.84%. In addition, races 001, 113, and 151 had respective magnitudes of 7.38%. Previous research by Utami *et al.* (2000) successfully grouped blast races based on their virulence. Group I, represented by race 001, is characterized as an avirulent, widely spread, and able to survive in the field. Group II, represented by race 033, was characterized by high variation in virulence and presumably able to adapt to selection pressure. Lastly, group III belongs to highly virulent races, but it cannot survive under field conditions and is represented by race 173.

Understanding the molecular mechanisms of plant-pathogen interactions and resistance gene introgression relies on the identification and isolation of resistant genes from host plants and avirulent pathogenic genes (Vasudevan *et al*., 2015; Zhu *et al*., 2016; Ning *et al*., 2020; Kurrata *et al.*, 2021). Gene collections are currently characterized based on molecular markers to explore untapped allele resources. This technique is crucial for recognizing genes that confer broad-spectrum blast resistance and combating active blast races (Qu *et al*., 2006; Li *et al*., 2020; Sheoran *et al*., 2021).

Molecular marking aids in the detection and combination of multiple blast-resistance genes that confer durable resistance against various races and dynamic blast pathogens (Xiao *et al*., 2016; Orasen *et al*., 2020). Xu *et al.* (2014) and Ramkumar *et al.* (2015) conducted genetic studies on genes involved in virulence in *P. oryzae*. Several researchers have also identified blast resistance genes that control compatibility with certain varieties, genes that control blast development during infection, and genes that affect the formation and penetration of the appressorium (Vasudevan *et al*., 2015; Zheng *et al*., 2016; Xiao *et al*., 2017). The

genes that were successfully cloned were Pi37 in chromosome 1 (Lin *et al*., 2007), Pib in chromosome 2, Pi9 (Qu *et al*., 2006), Pid2 in chromosome 6 (Chen *et al*., 2006), and Pita in chromosome 12 (Bryan *et al*., 2000). Pup1 contains dirigent-like genes with -dioxygenase fatty acids and aspartate proteinases, which play important roles in producing proteins involved in lignin biosynthesis and eventually affect cell wall strength (Heuer *et al*., 2009). The fifteen blast-resistance genes (Pid2, Piz, Pizt, Pi9, Pi36, Pi37, Pi5, Pib, Pikp, Pikh, and Pita2) have been identified in rice through molecular marker screening for blast-resistance genes (Su *et al*., 2015; Yan *et al*., 2017; Jiang *et al*., 2019; Meng *et al*., 2020). This marking technique is extremely helpful for detecting several potential blast-resistance genes with durable resistance to identify pyramiding genes that are able to overcome multiple races and highly dynamic blast pathogens (Xiao *et al*., 2015; Orasen, *et al*., 2020; Wang *et al*., 2023).

Developing sustainable blast-resistant rice varieties with horizontal resistance is crucial. Developing blast-resistant plants through producing genotypes with multiple resistance genes that can conquer various dynamic blast races in farming areas is an effective strategy (Wang and Valent, 2017; Herawati *et al*., 2022). Several studies have successfully developed a pyramid program for long-term blast resistance (Xiao *et al*., 2016; Orasen *et al*., 2020; Mutiga *et al*., 2021).

The genetic diversity in germplasm sources significantly contributes to successful rice-breeding programs (Sitaresmi *et al*., 2013). Due to their natural resistance to environmental stressors, pests, and diseases, local plant varieties are highly valued as a collection of genetic resources (Khairullah *et al.,* 2021). Incorporating resistance genes to stressors into rice breeding can enhance the superiority of rice varieties (Nickolas *et al*., 2018; Yadav *et al*., 2019). Since 2015, upland rice breeding has incorporated Bugis and Sriwijaya landrace varieties and IR 7858-1/IR148 lines for blast disease resistance (Herawati *et al*., 2017; Herawati *et al.,* 2021). The research finding was identified blast resistance genes in upland rice inbred lines derived from crossing landrace varieties with site-specific blast races.

Materials and methods

Screening for blast resistance

Nineteen lines derived from crosses between Sriwijaya and Bugis varieties and drought-resistant lines (IR7858 and IR148+) were assessed for blast resistance. As a control variety, Situ Patenggang was used as a resistant check variety, whereas Kencana Bali was used as a susceptible check variety (Table 1,

data supplement). The materials used were four Pyricularia races obtained in previous experiments, i.e., races 373-BS and 333-BT representing high virulence races, race 043-RM representing moderate virulence races, and race 001-BU representing low virulence races. Isolates taken from previous experiments were propagated with PDA media for 4-5 days. The petri dishes with PDA medium were inoculated with fungal hyphae. The rice seedlings were sprayed uniformly with the spore suspension when they had three to four leaves (18 to 21 days old). 24 hours post-exposure, inoculated plants were moved to a screen house for humidity preservation. Disease scale observations were carried out 7 days after inoculation following the IRRI SES (IRRI, 2013) (Table 2, data supplement). The length of the latency period, the quantity, the percentage, and the severity of the lesions were all observed.

Determination of blast-resistant genes

Four specific primer pairs were used to detect multigenic blast resistance genes: (1) Pib (F: GACTCGGTCGACCAATTCGCC; R: ATCAGGCCAGGCCAGATTTG), (2) Pii (F: GGATGATGTGATCTGCAGAG; R: CTCTTGGTGATCTTTGTTAC), (3) Pi5 (F: GATATGGTTGAAAAGCTAATCTCA; R-ATCATTGTCCTTCATATTCAGAGT), and (4) Pita2 (F: AGCAGGTTATAAGCTAGGCC; R: CTACCAACAAGTTCATCAAA (Table 3, data supplement). 0.1 g of rice leaf samples were crushed using liquid nitrogen and total DNA was isolated following the Wizard Genomic DNA Purification Kit protocol. The sample was placed into a 2 mL Eppendorf tube, heated in a water bath at 65°C for 15 minutes, vortexed for 1-3 seconds, and 600 µL incubated at 37°C for 15 minutes. 200µl of Protein Precipitation Solution was added and centrifuged for 3 minutes at 13,000 rpm. In a 1.5 ml microtube, add 600µl of room-temperature isopropanol to the supernatant. The centrifugation of the solution lasted for 1 minute at room temperature. Allow the solution to dry for 15 minutes before continuing. Alternatively, incubate at 65°C with 100µl DNA Rehydration Solution for 1 hour, or store overnight at 4°C.

PCR was performed at 94°C for 5 minutes as a preliminary step, followed by 35 cycles consisting of denaturation at 94°C for 1 minute, annealing for 2 minutes, and extension at 72°C for 2 minutes, concluding with a final extension at 72°C for 10 minutes. DNA electrophoresis was performed on a 1.5% TBE agarose gel (0.6 g agarose in 1x40 mL TAE buffer) for 30 minutes at 100 V to assess amplification success. The DNA bands' dispersion was evaluated by observing the electrophoresis results under a UV transilluminator following a 10-minute EtBr soak, a 5-minute ddH2O rinse, and drying. Blast resistance genes

were identified when bands of a specific size were present in the lines tested for each primer.

Multigenic resistance genes in selected lines were identified through DNA sequencing using BigDye® Terminator First Base Services (Genetika Sains Indonesia publishes the scientific research). The alignment analysis was carried out using ClustalX and Mega X software version 10.2.6, while the sequencing results were edited with Bioedit software version 7.2. Amino acid analysis was performed using BLASTX from NCBI (www.ncbi.nlm.nih.gov/BLAST/). 1000 Neighbor-Joining trees were generated using MEGA X for phylogenetic construction.

Data analysis

The following formula developed by IRRI (2013) was used to assess disease severity:

$$DS = \frac{\sum_{i=0}^{n}(nixvi)}{N\ x\ Z}\ x\ 100\% \qquad (1)$$

where Disease severity is measured by DS; symptom criteria score is measured by v in the family i-th; number of families assaulted is measured by ni in the i-th score; number of clumps detected is measured by N; and highest score is measured by Z.

Results

Blast resistance in inbred rice lines

Screening of 19 evaluated lines inoculated with four local races (333-BT, 001-BU, 043-RM, and 373-BS) indicated variations in blast resistance. The latent period, as indicated by the appearance of lesions for the first time in susceptible varieties since inoculation, took less than 3 days for K. Bali, whereas for the resistant varieties, Salumpikit took 4-5 days since the first inoculation (Figure 1A). This finding was confirmed based on the number of lesions and percentage in the leaf (Figure. 1B, C). There were nine rice lines (G7, G8, G9, G11, G13, G14, G15, G18, and G19) that showed resistance to blast disease with lower severity (Figure 2). There are race-sensitive lines with high virulence (333-BT and 373-BS), namely G3, G4, G6, G12, and G17. In addition, races 333-BT and 373-BS tended to show high virulence in sensitive varieties of K. Bali. Almost all lines had low severity in the low-virulence races (001-BU and 043 RM). Overall, there was consistency in line resistances (based on the severity of the 19 upland rice lines), in which those of low virulence races tended to be more

resistant to almost all lines, and there were several lines that showed low severity in high virulence races.

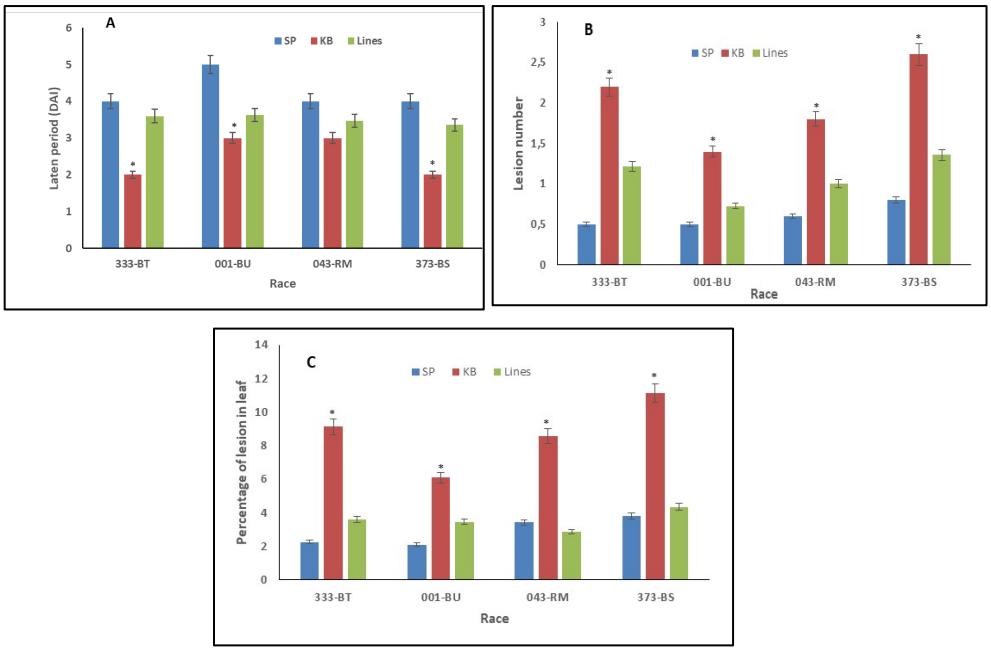

Figure 1. Screening blast resistance of upland rice lines under greenhouse conditions using races 333-BT, 001-BU, 043-RM, and 373-BS: (A) measuring the latent period, (B) number of lesions on leaves, and (C) percentage of lesions, with significant differences (*) in all variables between the tested lines and control varieties as determined by two-tailed Student's t-tests ($p < 0.05$)

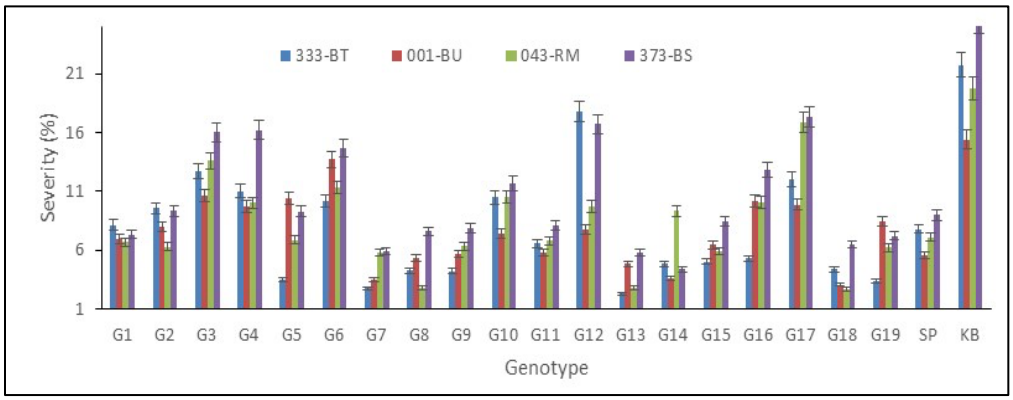

Figure 2. The severity of the 19 rice lines, including SP and KB as control varieties, were inoculated with 333-BT, 001-BU, 043-RM, and 373-BS

Detection of blast-resistant genes

The presence of the blast resistance gene was detected in 19 inbred lines resulting from single crosses of Bengkulu landraces using four specific primers (Table 3). Results concluded that all four primers could determine the presence of the Pib, Pii, Pi5, and Pita2 genes (Figure 3). The Pib gene was detected at 100% in the lines tested (388 bp), the Pii gene was detected in G5, G6, G8, G9, G11, G14, G15, G16, G17, G18, and G19, or 58% expressed at 484 bp, and the Pi5 gene was expressed in lines G14, G16, G17, and G19 (21%) with a size of 1450 bp. Meanwhile, 40% of the Pita2 gene was detected at 1042 bp in lines G7, G8, G9, G11, G13, G14, G15, and G18. Sequencing of lines carrying the Pii, Pi5, and Pita2 genes confer resistance to pathogens with high virulence.

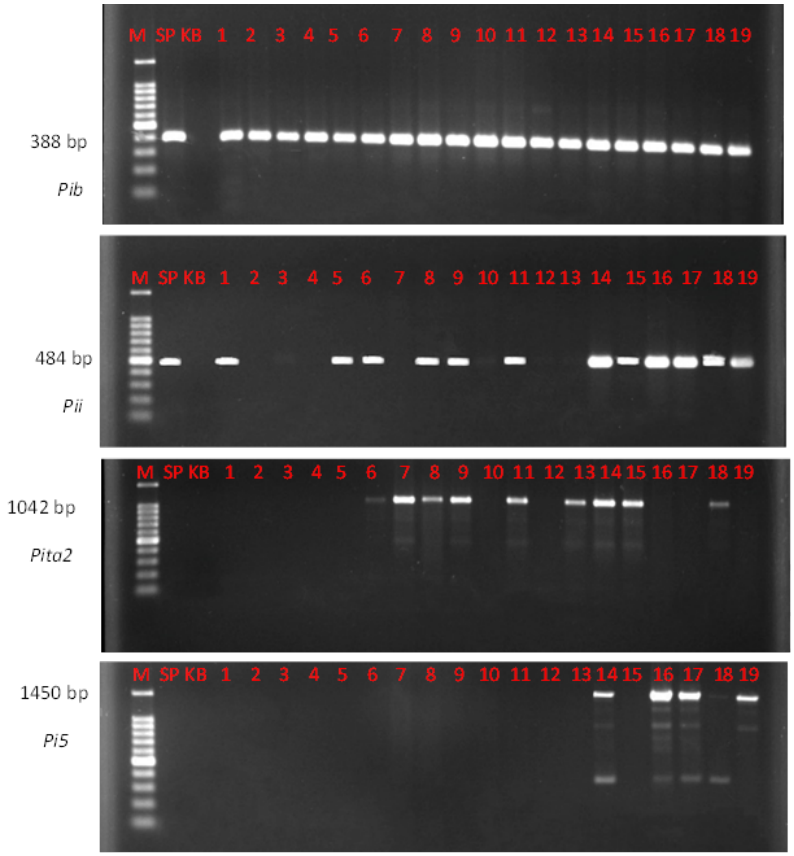

Figure 3. DNA amplification of the blast resistance genes Pib (388 bp), Pii (484 bp), Pita2 (1042 bp), and Pi5 in 19 selected rice lines with Situ Patenggang (SP) and Kencana Bali (KB) as positive and negative controls, respectively (M= DNA ladder of 100 kb)

Table 1. The homology of genes Pita2, Pi5, and Pii among rice lines was analyzed utilizing NCBI's BLASTX tool

Genotype	Homology	E-value	Identity	Accesion number
G8_Pi-ta, G9_Pi-ta, G10_Pi-ta, G12_Pi-ta, G14_Pi-ta, G15_Pi-ta, G16_Pi-ta, G19_Pi-ta	Oryza sativa Indica Group partial pi-ta gene for NBS-LRR, cultivar Vana surya, exons 1-3	0,00	99,90%	HE661565.1
	Oryza sativa Indica Group cultivar Amano Bavo Pi-ta gene, partial cds.	0,00	99,90%	GU269201.1
	Oryza sativa Indica Group partial pita gene for NBS-LRR, cultivar Parimala kalvi, exons 1-3	0,00	99,90%	HE589949.1
	Oryza sativa Indica Group partial pita gene for NBS-LRR, cultivar Thule atte, exons 1-3	0,00	99,90%	HE589953.1
	Oryza sativa cultivar Tijun NBS-LRR resistance protein (Pita) gene, partial cds	0,00	99,90%	JF897634.1
	Oryza sativa Indica Group cultivar Podumoni Ahu Pi-ta gene, partial cds.	0,00	99,80%	GU269203.1
	Oryza sativa blast resistance protein Pi-ta variant 11 gene, complete cds	0,00	99,90%	EU770217.1
	Oryza rufipogon Pi-ta gene, complete cds, strain: W1972.	0,00	99,80%	AB364482.1
	Oryza sativa Indica Group cultivar HPR-2178 NBS-LRR resistance protein (Pita) gene, partial cds.	0,00	99,80%	JN230597.1
	Oryza sativa cultivar Beesginsali NBS-LRR resistance protein (Pita) gene, partial cds.	0,00	99,80%	JF897635.1
G6_Pii, G9_Pii, G15_Pii, G18_Pii, G18_Pii, G20_Pii,	Oryza sativa cultivar Fujisaka 5 Pii-2 gene, complete cds.	0,00	99,79%	MH490983.1
	Oryza sativa Indica Group cultivar Shuhui498 chromosome 9 sequence.	0,00	99,79%	CP018165.1
	Oryza sativa 301246 QTL43_2-14 protein gene, complete cds.	0,00	99,79%	KM016820.1
	Oryza sativa 301278 QTL43_2-13 protein gene, complete cds.	0,00	99,79%	KM016819.1
	Oryza sativa cultivar Fujisaka 5 Pii-2 mRNA, complete cds.	0,00	99,79%	MH490981.1
	Oryza sativa 312007 QTL43_2-1 protein gene, complete cds.	0,00	99,22%	KM016807.1
	Oryza sativa Japonica Group Pii-2 mRNA for putative disease	0,00	99,22%	LC190730.1

Genotype	Homology	E-value	Identify	Accesion number
	resistance protein, complete cds, cultivar: Hitomebore.			
G16_Pi5, G17_Pi5, G19_Pi5	Oryza sativa Indica Group cultivar Shuhui498 chromosome 9 sequence.	0,00	99,41%	CP018165.1
	Oryza sativa Indica Group cultivar Minghui 63 chromosome 9.	0,00	99,41%	CP054684.1
	Oryza brachyantha ankyrin repeat-containing protein At5g02620-like (LOC102714950), mRNA.	0,004	83,18%	XM_015840816.1

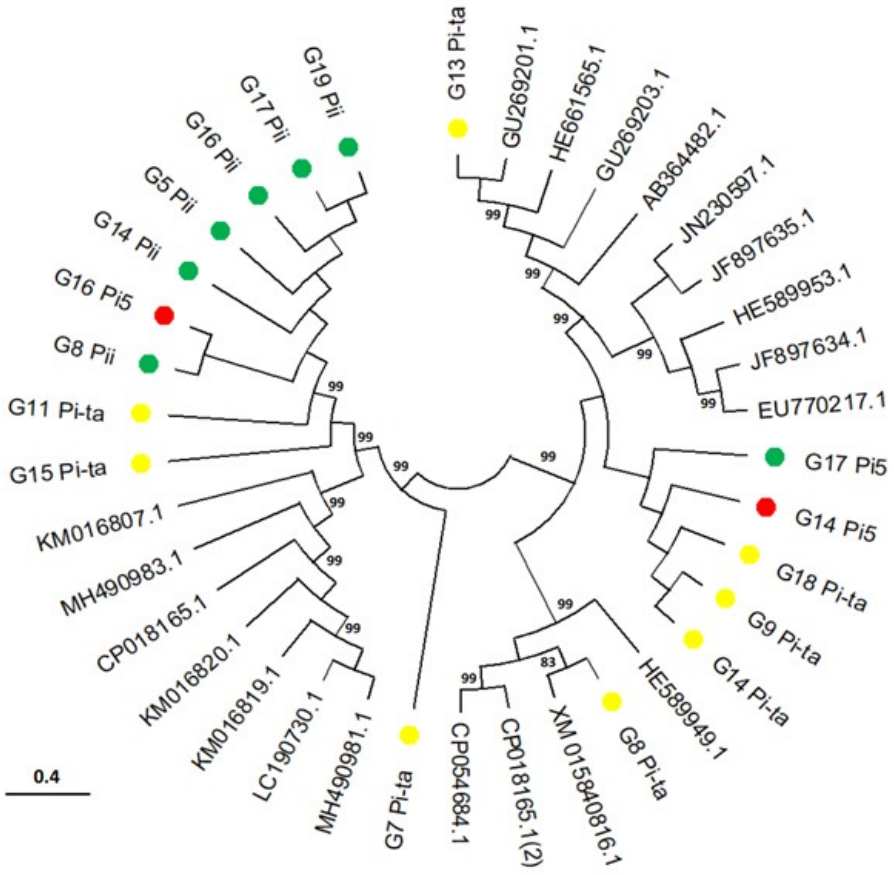

Figure 4. A neighbor-joining tree, built from amino acid sequences of rice lines identified by Pita2, Pii, and Pi-5 genes, was verified with 1000 bootstrap replications

Table 2. Interactions of the Pib, Pii, Pita2, and Pi5 genes with blast races of 001-BU, 043-RM, 333-BT, and 373-BS in 19 upland rice lines

Lines	Gene*				Race**			
	Pib	Pii	Pita2	Pi5	001-BU	043-RM	333-BT	373-BS
G1	+	+	-	-	R	R	MR	MR
G2	+	-	-	-	R	MR	MR	MR
G3	+	-	-	-	R	MR	MR	S
G4	+	-	-	-	R	MR	MR	S
G5	+	+	-	-	R	MR	R	MR
G6	+	+	-	-	R	MR	MR	S
G7	+	-	+	-	R	R	R	R
G8	+	+	+	-	R	R	R	R
G9	+	+	+	-	R	R	R	R
G10	+	-	-	-	R	MR	MR	MR
G11	+	+	+	-	R	R	R	R
G12	+	-	-	-	R	MR	S	S
G13	+	-	+	-	R	R	R	R
G14	+	+	+	+	R	R	R	R
G15	+	+	+	-	R	R	R	R
G16	+	+	-	+	R	MR	R	MR
G17	+	+	-	+	R	MR	MR	MR
G18	+	+	+	-	R	R	R	R
G19	+	+	-	+	R	R	R	R
KB	-	-	-	-	S	S	S	S
SP	+	+	-	-	R	R	R	R

*(+) gene present, (-) gene absent;**Scoring based on SES IRRI (2013) R: Resistance, MR: Moderately Resistance, S: Susceptible

The sequencing results were analyzed using the BLASTX (Basic Local Alignment Search Tools X) program with the amino acid sequences from NCBI to distinguish the homology of the amino acid sequences (Table 1). In 14 rice lines, the NBS-LRR-partial resistance protein, which encodes a protein with NLR domain nucleotide bonds, exhibited the highest similarity (99%) to the amino acid sequences of the Pita2, Pii, and Pi5 genes. Other homologs included the Oryza sativa Japonica Group Pii-2 putative disease-resistant protein with 99% similarity and the Pi5 Oryza sativa Indica Group cultivar Shuhui498 with the chromosome 9 sequence. High homology, with a minimum of 100 base pairs and at least 70% base sequence or 25% amino acid sequence identity, indicates precise gene sequencing. The E-value of nucleotide binding site-leucine rich

repeat (NLR) domains in rice lines from GenBank (www.ncbi.nlm.nih.gov) represents the statistical probability of sequence similarity. All accessions in the gene bank recorded a value of 0 for the tested sequences in Table 1. According to phylogenetic analysis using the neighboring joining method, rice lines with Pita2, Pi5, and Pii genes formed three similar groups, and all tested lines were closely related to their respective homologous accession numbers (Figure 4).

Discussion

19 lines' blast resistance levels varied in the screening results. The latent period of sensitive variety K. Bali was less than 3 days. In K. Bali, it takes 4-5 days to grow the resistant Salumpikit variety. Result provided the number and percentage of lesions. Blast disease poses a significant threat to susceptible plants. High virulence races, such as 373 and 333, cause susceptibility in lines like G3, G4, G6, G12, and G17, while low virulence races, including 001 and 043, induce resistance in almost all other lines. Several rice lines showed resistance to all the tested races, including G7, G8, G9, G11, G13, G14, G15, G18, and G19.

The determination of blast resistance genes showed that four specific primers could detect the genes Pib, Pii, Pi5, and Pita2. Pib was detected at 100% in the tested lines (388 bp), whereas Pii was detected in G5, G6, G8, G9, G11, G14, G15, G16, G17, G18, and G19, representing 58% expressed in 484 bp. Meanwhile, the Pita2 gene was 40% detected at 1042 bp in lines G7, G8, G9, G11, G13, G14, G15, and G18, and the Pi5 gene was expressed in lines G14, G16, G17, and G19. Interestingly, it appeared that the presence of this gene was related to the virulence of the pathogen, in which plants that were detected to have the Pib gene are able to overcome attacks by pathogens with low virulence, while Pii is able to control attacks by pathogens with moderate virulence and is expressed in 58% of lines. Pi5 and Pita2 were only detected in a few lines, indicating that the plant was able to overcome pathogens with high virulence. It was previously known that the Pita2 gene conferred broad-spectrum blast resistance and that its location was in the centromere region on chromosome 12 (Meng *et al*., 2020; Herawati *et al*., 2022; He *et al*., 2022).

The cellular development and morphology of *P. oryzae* are very adaptive to the infected rice plants. It has been reported that this pathogenic fungus has high genetic diversity (Longya *et al*., 2020; Sheoran *et al*., 2021). Therefore, it is crucial to create rice varieties that are blast-resistant and horizontally resistant. Creating genotypes through the pyramiding of resistance genes or the use of multiple resistance genes is one method for creating blast-resistant varieties that can be used to combat various blast races that might emerge in rice fields. A

pyramid program has been successfully used by researchers to induce long-lasting blast resistance (Xiao *et al*., 2016; Orasen *et al*., 2020)

As suggested by the sequence analysis of BlastX, 10 gene homologs from eight rice lines detected by Pita2 and seven gene homologs from six lines detected by Pii were found in the gene bank. Among the Pita2 gene sequences discovered in eight rice lines, the NBS-LRR protein from the Indica group of Oryza sativa showed the highest 99% similarity with the probable disease resistance Pii mRNA gene. These results are consistent with the phylogenetic analyses, which revealed a very close relationship between homologous gene groups and the rice lines tested. Through pathogen recognition, plants produce resistance proteins (R) in response to their activation of hypersensitive defense mechanisms. (Takken *et al*., 2006). The NBS-LRR resistance gene, which is the most common family of plant resistance genes (Meyers *et al*., 1999; Nimchuk *et al*., 2003), encapsulates the most significant plant resistance gene. Resistance genes generally have a structure consisting of an N-terminal nucleotide binding (NB) domain and C-terminal leucine-rich repeats (LRRs) (Seong *et al*., 2020 and Maruta *et al*., 2022).

Interactions of Pib, Pii, Pita2, and Pi5 genes with blast races 001, 043, 333, and 373 in 19 upland rice lines are demonstrated. Resistance to race 001 is conferred by all lines via the Pib gene. According to Amir *et al.* (2000), four races (001, 003, 033, and 173) exist seasonally. These isolates are widely distributed. Isolates of race 001 have the lowest pathogenicity level of virulence, spread widely, and can survive long in the field (Utami *et al*., 2000). The Pii gene is not expressed in all lines, but expression of this gene was observed in 001, 043, and 333, which were moderately resistant to resistance. In all tested races - G7, G8, G9, G11, G13, G14, G15, and G18 - resistance was present due to the Pita2 gene. A recent study revealed that Pita's Pita2 encodes an R protein identical to the previously cloned Ptr (Meng *et al*., 2020; Herawati *et al*., 2022). The study revealed that Pita2 confers resistance to all avr-Pita races in the multigene-detected lines, including those in lines G7, G8, G9, G11, G13, G14, G15, G18, and G19. Therefore, blast-resistant rice varieties with long-term and polygenic resistance are essential. One of the strategies to overcome multi-racial blast pathogens is to develop blast-resistant varieties that have many resistance genes through pyramiding genes (Xiao *et al*., 2016; Orasen *et al*., 2020).

Information on the geographical composition and dominance of blast disease pathogenic races in particular rice ecosystems is important as a basis for determining disease control strategies using resistant varieties. The suitability of planting resistant varieties with a composition of pathogenic races in the field would increase the effectiveness of blast disease control and eventually suppress disease transmission. Furthermore, farmers can combat blast disease by planting

resistant cultivars in accordance with the available races in each location, using information on the distribution of blast races as a guide. In this study, we found that the G7, G8, G9, G11, G13, G14, G15, G18, and G19 lines have the potential to be further evaluated because they contain the Pii, Pi5, and Pita2 genes, which have multigenic and broad-spectrum blast resistance.

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References

Amir, M., Nasution, A. and Santoso. (2000). Inventory of *Pyricularia oryzae* races in Sukabumi area, West Java, growing season 1995-1998. Prosiding Nasional XV dan Seminar Ilmiah PFI 16-18 September 1999. UNSOED. Purwokerto.

Bryan, G. T., Wu, K. S., Farrall, L., Jia, Y., Hershey, H. P., McAdams, S. A., Faulk, K. N., Donaldson, G. K., Tarchini, R. and Valent, B. (2000). A single amino acid difference distinguishes resistant and susceptible alleles of the rice blas resistance gene Pi-Ta. The Plant Cell, 12:2033-45.

Chen, X., Shang, J., Chen, D., Lei, C., Zhou, Y., Zhai, W., Liu, G., Xu, J., Ling, Z., Cao, G., Ma, B., Wang, Y., Zhao, X., Li, S. and Zhu, L. (2006). A B-lectin receptor kinase gene conferring rice blas resistance. The Plant Journal, 46:794-804.

He, N., Huang, F., Yu, M., Zhu, Y., Li Q. Q. and Yang, D. (2022). Analysis of a rice blast resistance gene pita-fuhui2663 and development of selection marker. Scientific Reports, 12:1-10.

Herawati, R., Inoriah, E., Rustikawati. and Mukhtasar. (2017). Genetics diversity and agronomic characters of F3 lines selected by recurrent selection for drought tolerance and blast resistance of Bengkulu local rice varieties. International Journal on Advanced Science, Engineering and Information Technology, 7:922-27.

Herawati, R., Herlinda, S., Ganefianti, D.W., Bustamam, H. and Sipriyadi. (2022). Improving broad spectrum blast resistance by introduction of the Pita2 gene: encoding the NB-ARC domain of blast-resistant proteins into upland rice breeding programs. Agronomy, 12: 2373.

Herawati, R., Lestari, A. P., Ganefianti, D. W. and Romeida, A. (2021). Comparative study on the stability and adaptability of different models to develop a high-yield inbred line from landrace rice varieties. Annals of Agricultural Sciences, 66:184-92.

Heuer, S., Lu, X., Chin, J. H., Tanaka, J. P., Kanamon, H., Matsumoto, T., Leon, T. D., Ulat, V. J., Ismail, A. M., Yano, M. and Wissuwa, M. (2009). Comparative sequence analyses of the major quantitative trait locus phosphorus uptake 1 (Pup1) reveal a complex genetic structure. Plant Biotechnology Journal, 7:456-71.

International Rice Research Institute. (2013). Standard evaluation system for rice. The International Rice Testing Program (IRTP) IRRI Los Banos, Philippines. Retrieved (http://www.clrri.org/ver2/uploads/SES_5th_edition.pdf).

Jiang, H., Li, Z., Liu, J., Shen, Z., Gao, G., Zhang, Q. and He, Y. (2019). Development and evaluation of improved lines with broad-spectrum resistance to rice blast using nine resistance genes. Rice, 12:1.

Khairullah, I., Saleh, M. and Mawardi. (2021). The Characteristics of Local Rice Varieties of Tidal Swampland in South Kalimantan. IOP Conference Series: Earth and Environmental Science, 762:1. doi: 10.1088/1755-1315/762/1/012009.

Kurrata, G., Kuswinanti, T. and Nasruddin, A. (2021). Keparahan penyakit blas *Pyricularia oryzae* dan analisis gen virulensi menggunakan metode sequence characterized amplified region. Jurnal Fitopatologi Indonesia, 17:19-27.

Li, W., Deng, Y., Ning Y., He, Z. and Wang, G. L. (2020). Exploiting broad-spectrum disease resistance in crops: from molecular dissection to breeding. Annual Review of Plant Biology, 71:575-603.

Lin, F., Chen, S., Que, Z., Wang, L., Liu, X. and Pan, Q. (2007). The blas resistance gene Pi37 encodes a nucleotide binding site leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. Genetics, 177:1871-80.

Liu, T., Wan, A., Liu, D. and Chen, X. (2017). Changes of races and virulence genes in *Puccinia striiformis* f. Sp. Tritici, the wheat stripe rust pathogen, in the United States from 1968 to 2009. Plant Disease, 101:1522-32.

Longya, A., Talumphai, S. and Jantasuriyarat, C. (2020). Morphological characterization and genetic diversity of rice blast fungus, *Pyricularia oryzae*, from Thailand using ISSR and SRAP Markers. Journal of Fungi, 6:1.

Maruta, N., Burdett, H., Lim, B. Y. J., Hu, X., Desa, S., Manik, M. K. and Kobe, B. (2022). Structural basis of NLR activation and innate immune signalling in plants. Immunogenetics, 74:5-26.

Meng, X., Xiao, G., Telebanco-Yanoria, M. J., Siazon, P. M., Padilla, J., Opulencia, R., Bigirimana, J., Habarugira, G., Wu, J., Li, M., Wang, B., Lu, G. D. and Zhou, B. (2020). The Broad-spectrum rice blast resistance (R) gene Pita2 encodes a novel R protein unique from Pita. Rice, 13:1.

Meyers, B. C., Dickerman, A. W., Michelmore, R. W., Sivaramakrishnan, S., Sobral, B. W. and Young, N. D. (1999). Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant Journal. 20:317-32.

Mulyaningsih, E. S., Perdani, A. Y., Indrayani, S. and Suwarno (2016). Seleksi fenotipe populasi padi gogo untuk hasil tinggi, toleran alumunium dan tahan blas di tanah masam. Jurnal Penelitian Pertanian Tanaman Pangan, 35:191.

Mutiga, S. K., Rotich, F., Were, V. M., Kimani, J. M., Mwongera, D. T., Mgonja, E., Onaga, G., Konate, K., Razanaboahirana, C., Bigirimana, J., Ndayiragije, A., Gichuhi, E., Yanoria, M. J., Otipa, M., Wasilwa, L., Ouedraogo, I., Mitchell, T., Wang, G. L., Correll, J. C. and Talbot, N. J. (2021). Integrated strategies for durable rice blast resistance in Sub-Saharan Africa. Plant Disease, 105:1-22.

Nickolas, H., Jayalekshmy, V. G., Yamini Varma, C. K. and Vighneswaran, V. (2018). Molecular and field level screening for blast resistance gene donors among traditional rice varieties of Kerala. Journal of Tropical Agriculture, 56:93-98.

Nimchuk, Z., Eulgem, T., Holt, B. F. and Dangl, J. L. (2003). Recognition and response in the plant immune system. Annual Review of Genetics, 37:579-609.

Ning, X., Yunyu, W. and Aihong, L. (2020). Strategy for use of rice blast resistance genes in rice molecular breeding. Rice Science, 27:263-77.

Orasen, G., Stile, M. R., Greco, R., Puja, E. and Pozzi, C. (2020). Blast resistance R genes pyramiding in temperate japonica rice. Euphytica, 216:40.

Qu, S., Liu, G., Zhou, B., Bellizzi, M., Zeng, L., Dai, L., et al. (2006). The Broad-spectrum blast resistance gene Pi9 encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. Genetics, 172:1901-1914.

Ramkumar, G., Prahalada, G. D., Hechanova, S. L., Vinarao, R. and Jena, K. K. (2015). Development and validation of SNP-based functional codominant markers for two major disease resistance genes in rice (O. Sativa L.). Molecular Breeding, 35:129.

Seong, K., Seo, E., Witek, K., Li, M. and Staskawicz, B. (2020). Evolution of NLR resistance genes with noncanonical N-terminal domains in wild tomato species. New Phytologist, 227:1530-43.

Sheoran, N., Ganesan, P., Mughal, N. M., Yadav, I. S. and Kumar, A. (2021). Genome assisted molecular typing and pathotyping of rice blast pathogen, *Magnaporthe oryzae*, reveals a genetically homogenous population with high virulence diversity. Fungal Biology, 125:733-47.

Sitaresmi, T., Wening, R. H., Rakhmi, A. T., Yunani, N. and Susanto, U. (2013). Pemanfaatan plasma nutfah padi varietas lokal dalam perakitan varietas unggul. Iptek Tanaan Pangan, 8:22-30.

Su, J., Wang, W. J., Han, J. L., Chen, S., Wang, C. Y., Zeng, L. X., et al. (2015). Functional divergence of duplicated genes results in a novel blast resistance gene Pi50 at the Pi2/9locus. Theoretical and Applied Genetics, 128:2213-2225.

Sudir., Nasution, A. and Nuryanto, B. (2014). Penyakit blas *Pyricularia grisea* pada tanaman padi dan strategi pengendaliannya. IPTEK Tanaman Pangan, 9:85-96.

Takken., Frank, L. W., Albrecht, M. and Tameling, W. I. L. (2006). Resistance proteins: molecular switches of plant defence. Current Opinion in Plant Biology, 9:383-90.

Utami, D. W., Amir, M. and Moeljopawiro, S. (2000). Analisis RFLP kelompok ras dan haplotipe isolat blas dengan DNA pelacak MGR 586. Jurnal Bioteknologi Pertanian, 5:28-23.

Vasudevan, K., Gruissem, W. and Bhullar, N. K. (2015). Identification of novel alleles of the rice blast resistance gene Pi54. Scientific Reports, 5:1-12.

Wang, G. L. and Valent, B. (2017). Durable resistance to rice blast. Science, 355:906-7.

Wang, Y., Tang, S., Guo, N., An, R., Ren, Z., Hu, S., Wei, X., Jiao, G., Xie, L., Wang, L., Chen, Y., Zhao, F., Tang, S., Hu, P. and Sheng, Z. (2023). Pyramiding rice blast resistance gene Pi2 and fragrance gene Badh2. Agronomy, 13:1-14.

Xiao, N., Wu, Y., Pan, C., Yu, L., Chen, Y., Liu, G., Li, Y., Zhang, X., Wang, Z., Dai, Z., Liang, C. and Li, A. (2017). Improving of rice blast resistances in japonica by pyramiding major R genes. Frontiers in Plant Science, 7:1-10.

Xiao, W. M., Yang, Q. Y., Sun, D. Y., Wang, H., Guo, T., Liu, Y. Z., Zhu, X. Y. and Chen, Z. Q. (2015). Identification of three major R genes responsible for broadspectrum blast resistance in an indica rice accession. Molecular Breeding, 35:49.

Xiao, W., Luo, L. X., Wang, H., Guo, T., Liu, Y. Z., Zhou, J. Y., Zhu, X. Y., Yang, Q. Y. and Chen, Z. Q. (2016). Pyramiding of Pi46 and Pita to improve blast resistance and to evaluate the resistance effect of the two R genes. Journal of Integrative Agriculture, 15:2290-98.

Xu, X., Hayashi, N., Wang, C.T., Fukuoka, S., Kawasaki, S., Takatsuji, H. and Jiang, C. J. (2014). Rice blast resistance gene pikahei-1(t), a member of a resistance gene cluster on chromosome 4, encodes a nucleotide-binding site and leucine-rich repeat protein. Molecular Breeding, 34:691-700.

Xu, X., Tang, X., Han, H., Yang, W., Liu, X., Li, K., Tan, Y., Qin, Y., Liu, X. and Wang, C. (2021). Pathogenicity, mating type distribution and avirulence gene mutation of *Magnaporthe oryzae* populations in the wuling mountain region of China. Physiological and Molecular Plant Pathology, 116:101716.

Yadav, M. K., Aravindan, S., Ngangkham, U., Raghu, S., Prabhukarthikeyan, S. R., Keerthana, U., et al. (2019). Blast resistance in indian rice landraces: genetic dissection by gene specific markers. PLoS ONE, 14:e0211061.

Yan, L., Bai-Yuan, Y., Yun-Liang, P., et al. (2017). Molecular screening of blast resistance genes in rice germplasms resistant to *Magnaporthe oryzae*. Rice Science, 24:41-47.

Zheng, W., Wang, Y., Wang, L., Ma, Z., Zhao, J., Wang, P., et al. (2016). Genetic mapping and molecular marker development for Pi65(t), a novel broad–spectrum resistance gene to rice blast using next–generation sequencing. Theoretical and Applied Genetics, 129:1035-1044.

Zhu, D., Kang, H., Li, Z., Liu, M., Zhu, X., Wang, Y., et al. (2016). A Genome–wide association study of field resistance to *Magnaporthe oryzae* in rice. Rice 9:44.

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