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RESEARCH ARTICLE

Molecular characterization of rice blast fungus (*Pyricularia oryzae*) from West Sumatra and their virulence to several rice cultivars

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Abstract

Global rice production is severely impacted by rice blast disease, which is a devastating condition caused by the fungal pathogen *Pyricularia oryzae*. Many countries have reported blast diseases associated with rice yield loss. In Indonesia, this disease also destroys rice production in several rice fields in West Java, East Java, Central Java, Sumatra, and Sulawesi. The objectives of this study were to identify rice blast fungal isolates in West Sumatra and assess the resistance level of eight local and modern rice genotypes to this disease. Additionally, the rice genotypes were also evaluated for the presence of *Pi* genes associated with blast resistance by utilizing 25 simple sequence-repeats (SSR) markers known to be linked to rice blasts. The seedlings of all rice genotypes were exposed to fungal isolates to determine their blast resistance capacity based on leaf morphological traits. Before conducting blast resistance testing, the local isolates of rice blast fungus were molecularly identified by aligning sequences in a pairwise comparison. Based on the identification process, three *P. oryzae* (MoK19-49, MoK19-45, and MoK19-28) fungal isolates were identified. The confirmed *P. oryzae* isolate MoK19-28 was then used to assess the blast resistance of the rice genotypes using the SES-blast test. The resistance levels of all genotypes were evaluated by observing the morphology of infected leaves for 24 days. Four genotypes, namely Cantik Manis (CM), Bungo Sungkai (BS), Inpari 48 Blas (IB) and Inpago 9 (Ip9), exhibited resistance to leaf blast caused by *P. oryzae* isolate MoK19-28. Meanwhile, Pulau Batu (PB), Mundam Putiah (MP) and IR64-*Sub1* (IR) displayed intermediate resistance to the fungus. In contrast, the blast fungus *P. oryzae* isolate MoK19-28 severely impacted the Kuriak (K) genotype. The rice variety IB contains several *Pi* genes, specifically *Pi1*, *Pi9*, *Pikh* and *Pi37*, which are associated with enhanced resistance to leaf blast caused by *P. oryzae* isolate MoK19-28. This genetic characteristic sets it apart from susceptible genotypes. This work has successfully identified multiple blast-resistant rice genotypes that could potentially be used in future rice improvement initiatives.

Keywords

molecular analysis; *Pyricularia oryzae*; *Pi* genes; rice blast resistance

Introduction

The biotic factor has a significant impact on rice productivity around the world. The Ascomycetes fungus *Pyricularia oryzae* causes blast disease, which is one of the most severe diseases that affect rice fields in Asia, Africa, Europe, and America (1). The majority of blast fungus migrations begin in Asia. The diversity of this fungus was found in Western Nepal and the Himalayan foothills (South China, Laos, North Thailand) (2). Despite the availability of resistant rice varieties, this fungus remains challenging to control. Therefore, identifying host-resistant types is crucial for the cost-effective eradication of blast diseases (3). When a hemibiotrophic fungus like *P. oryzae* infects a rice plant, it sends molecular and hormonal signals that help the plant's immune system work together. Initially, the plant activates salicylic acid (SA) signaling to combat the biotrophic phase. As the fungus shifts to its necrotrophy state, the plant modulates its defenses, increasing jasmonic acid (JA) and ethylene (ET) signaling while balancing reactive-oxygen species (ROS) production and crosstalk between different pathways. The ability of the plant to effectively respond to each phase of the fungus's life cycle (biotrophic and necrotrophic) depends on the intricate interplay between these signaling networks (4-6).

Resistance genes (R genes) are crucial components of the immune system. These genes encode proteins that can recognize specific pathogen-derived molecules (effectors) and trigger a strong immune response. This often leads to localized cell death (hypersensitive response) to stop the pathogen from spreading. When R proteins are activated, they initiate effector-trigger immunity (ETI), localized cell death, ROS production and the activation of defense genes. Therefore, identifying resistance genes is essential and we can use molecular methods to investigate the genetic basis of resistance to various crop diseases. This includes studying the impact of target crops, the resistance found in wild hosts or cultivars, the natural changes that occur in pathogen populations, how plants and pathogens interact, and markers for genotypes that carry resistance genes. Researchers have used molecular markers to identify hundreds of blast resistance genes (*Pi* genes) and QTLs in rice (7). *Pi1, Pik, Pikh, Pikm*, and *Pish* are among the broad-spectrum resistance genes that constitute the majority of *Pi* genes. These genes possess a strong resistance against numerous strains of the blast pathogen (8). Furthermore, *Pi37*, encoding a 1290 peptide NBS-LRR (nucleotide-binding site leucine-rich repeat), had been revealed to confer complete resistance to *P. oryzae* (9, 10), whereas *Pish* offers partial resistance to rice blast isolates (11). Furthermore, *Pik* offers comprehensive resistance to rice blast isolates (12). Additional R genes, including *Pi9, Pi39, Pi40*, and *Pita*, have demonstrated extensive resistance to blast illness. *Pita*'s protein, unlike *Pib* and other NBS-LRR genes, lacks a conventional LRR in its C-terminal domain. A leucine-rich domain (LRD) is made of 10 repeating structures of different lengths (from 16 to 75 amino acids) that are very badly designed (13, 14).

The chromosomal positions of the R genes and selection markers have been reported in numerous rice cultivars. Most of these R genes are found on chromosomes 1, 6, 11 and 12. At least 19 genes have been identified on chromosome 6. Furthermore, no less than 30 and 23 blast resistance genes have been found on chromosomes 11 and 12, respectively (15). DNA sequencing has become a popular method for identifying organisms in recent years. Through sequencing, researchers can identify numerous genes of interest, regulatory elements, and mutation sites. Furthermore, it is possible to determine species or population evolution by comparing homologous DNA sequences. In short, DNA sequencing can help discover gene alterations that may cause diseases (16). To date, there has been relatively little research on blast fungus detection in West Sumatra and its effects on local rice varieties. Therefore, this study aims to identify the leaf blast fungal isolates obtained from West Sumatra and determine their effects on several local rice cultivars grown by local farmers.

Materials and Methods

Eight rice genotypes were used in this study, which consisted of five landraces originating from West Sumatra and three modern cultivars. This study used a blast-resistant rice genotype, Inpari 48 Blas (IB), as a resistant check. The other two modern cultivars were Ip9 and IR. The details of the rice genotypes are presented in Table 1.

Blast fungus (P. oryzae) isolation and identification

Blast fungal samples were isolated from infected leaves collected from five rice fields in West Sumatra, Indonesia. The locations represented rice production center in the middle of the western coast of Sumatra, which is located at 0°54' N, 3°30' S latitude and 98°36' E, 101°53' E longitude. These rice cultivation areas had been reported to experience rice yield loss due to blast diseases. The infected leaves were characterized by dark-brown specks or spots on the leaf surface, particularly in the marginal and apical areas (Fig. 1). The infected leaves were cut before being taken to the laboratory and were placed in sterilized and labelled plastic bags for further culture and identification. The fungal inoculum was prepared following the procedure by (17). The fungal cultures were grown on Potato Dextrose Agar (PDA) medium and the fungal isolates were identified morphologically based on their hyphae characteristics. From 12 microbial isolates, four were chosen for further identification using

Table 1. The list of rice genotypes used as sample in the study

No.	Genotype	Rice type	Description
1.	Pulau Batu (PB)	Indica	Local rice, landrace, good-taste, high-yield
2.	Cantik Manis (CM)	Indica	Local rice, landrace, high-density grain
3.	Bungo Sungkai (BS)	Indica	Local rice, landrace, strong stem, many productive tillers, fast-harvested
4.	Kuriak (K)	Indica	Local rice, landrace, good taste
5.	Mundam Putiah (MP)	Indica	Local rice, landrace, good texture and taste
6.	Inpari 48 Blas (IB)	Indica	Modern rice, blast-resistance check, high-yield
7.	IR64-*Sub1* (IR)	Indica	Modern rice, short-stemmed, submergence tolerant
8.	Inpago 9 (Ip9)	Japonica	Modern rice, highland cultivar, blast-resistant

Fig. 1. Rice leaves infected by *P. oryzae* in different of severity: (A) healthy leaf/resistant; (B) 1 or 2 brown small spots appeared on particular leaf area; (C) brown spots/specks spread almost on all leaf areas

molecular techniques (DNA sequencing) using internal transcribed spacer/ITS region (18). The remaining eight microbial isolates did not exhibit the morphological characteristics of *P. oryzae*.

The DNA of the blast fungus was isolated following the protocol provided by GeneJET Genomic DNA Purification Kit (Thermofisher, USA). All PCR reactions were performed in a Biometra T3000 Thermal Cycler (by Used Lab Machines Ltd.). Briefly, primers internal transcribed spacer/ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') were used for PCR amplification. Each PCR reaction mixture contained 25 µL of Taq PCR Master Mix (Qiagen Inc.), 1 µL of each primer (10 µM), about 20 ng of fungal genomic DNA, and distilled water to make a final volume of 50 µL. PCR analysis was performed using the following conditions: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 1 min, and final extension at 72 °C for 5 min (19). The amplification products were resolved in 1.5 % agarose gel using 0.5X TBE buffer at 100 V for 45 min, followed by DNA staining with Ethidium Bromide and analysis using a Gel Doc XR+ with Image Lab software (Bio-Rad, Hercules, CA USA). PCR products were purified using the GF-1 PCR Clean-Up Kit (Vivantis). The sequence identity of the PCR product was determined by a pairwise alignment of two or more sequences with targeted sequence's full-length being 567 bp (20).

Bioinformatics analysis of blast fungus

The resulting sequences obtained from the fungal isolates were refined using BioEdit sequence alignment tools. The identification of each sequence was ascertained by a BLAST search conducted in the NCBI database. In order to determine the relationships between isolates, the sequences of all isolates in this study were aligned with the sequences of six reference *P. oryzae* isolates from various hosts (obtained from the GenBank) using the Clustal W algorithm in the MEGA X software version 11 (20). Moreover, a phylogenetic tree was constructed using the Neighbor-Joining method (21).

Identification of blast resistance genes (Pi) in rice genotypes

The leaf samples from 14 day old seedlings of each rice genotype were collected for DNA extraction. Genomic DNA was extracted using the Geneaid™ Genomic DNA Mini Kit (Geneaid Biotech Ltd.) according to the manufacturer's instructions. The DNA concentration was measured using the BioDrop™ spectrophotometer nanodrop, diluted at 25 ng/uL and stored at -20 °C. A total of 25 simple sequence repeats (SSRs) markers were obtained from the GRAMENE database, and the previous report of rice Insertion-deletion (InDel), single nucleotide polymorphism (SNP), and specific marker development (22-27). These markers were used to determine the presence of *Pi* genes in the tested rice genotypes (Table 1). A BLAST search was used to determine regions of similarity and verify the specificity of the markers for the Pi target region. PCR amplification was carried out using KOD OneTM PCR Master Mix-blue (Toyobo) with the following program: 94 °C for 5 min (initial denaturation), followed by 35 cycles at 94 °C for 30 s of denaturation, annealing at 48, 51, 55, 56, 60 and 62 °C for 30 s (annealing temperature depending on the marker's type), continuing extension at 72 °C for 30 s, and final extension at 72 °C for 7 min. PCR products with selected markers were analysed with 2 % agarose gel for electrophoresis.

Genetic structure analysis

The genetic structure of parental lines was analysed using STRUCTURE and the results were illustrated with the STRUCTURE Harvester Program (28).

P. oryzae inoculation and blast resistance screening

The confirmed *P. oryzae* isolates were then used to test the blast resistance of the rice plants. A ten day old fungal isolate was prepared for inoculation following the Standard Evaluation System (SES)-blast test by IRRI (29, 30). Rice seedlings (21 day old) were spray-inoculated with 25 mL *P. oryzae* spore suspension (1.5×10^5 spores/mL) of individual isolates (inoculum preparation provided in supplementary data). The inoculated plants were kept in a glasshouse under a 12 h day/12 h night photoperiod and 90 % relative humidity for 24 days. One week before assessing the plant's reaction, the plants were transferred to the incubation chamber set at 25 °C ± 2 °C. The lesions were scored according to the leaf blast scoring standards by IRRI (30), using a 0-9 SES scale: scores of 0-3 indicate resistance, 4-6 indicate moderate resistance and 7-9 indicate susceptibility.

Results

Fungus isolation and morphological identification

When plants are infected by blast fungus, brown specks appear on the leaf surface, starting from the leaf tips, margins, or both, and then spreading toward the outer edges. Immature lesions exhibit a pale green to greyish-green color, which gradually turns to yellow, light brown, and eventually grey (indicating death) with time. In susceptible genotypes, the lesions can spread across the entire length of the leaf and into the leaf sheath. Morphologically, blast fungus was characterized by white,

Table 2. Molecular screening results of eight rice genotypes using 25 DNA markers linked to *Pi* genes

No.	Gene	Marker	Expected amplicon size (bp)	Targeted band existence in each genotype							
				PB	CM	BS	K	MP	IB	IR	Ip9
1.	*Pi1*	RM1233	174	-	-	-	-	-	+	-	+
2.		RM224	157	-	-	-	+	-	+	+	-
3.		RM5926	150	-	-	-	+	-	+	+	-
4.	*Piz5*	RM527	233	-	-	+	-	+	+	-	+
5.	*Piz(t)*	Zt5659-1	257	+	+	+	+	+	+	+	+
6.	*Pi9*	195R-1	2000	-	-	-	-	-	+	-	-
7.		RM541	158	-	-	-	-	-	-	-	-
8.	*Pish*	OSR-3	150	+	+	+	+	+	+	+	+
9.		RM6648	207	-	-	-	-	-	+	-	+
10.		RM5811	97	+	+	+	+	+	+	+	+
11.	*Pikh/Pi54*	RM2191	237	-	-	-	-	-	-	-	-
12.		RM206	147	-	-	-	-	-	+	-	-
13.		RM144	247	-	-	-	-	-	+	-	-
14.	*Pik*	k-6816	218	-	-	-	+	-	-	-	-
15.	*Pikm*	Ckm-1	213	-	-	-	+	-	+	-	-
16.	*Pita/ Pita2*	YL155/YL87	1042	+	+	-	+	-	+	+	+
17.		OSM89	296	+	+	+	+	+	+	+	+
18.	*Pikh*	TRS26	200	-	+	-	+	+	-	-	+
19.	*Pib*	Pb28	388	+	+	+	+	+	+	+	+
20.		RM208	179	+	+	+	+	+	+	+	+
21.		RM166	316	+	+	+	+	+	+	+	+
22.	*Pi37*	RM302	156	-	-	-	-	-	+	-	-
23.		RM212	136	-	-	-	-	-	+	-	-
24.	*Pi39*	39SM	330	-	+	+	+	+	+	+	-
25.	*Pi40*	9871.T7E2b	642	-	+	+	+	+	+	+	-

compact, or cottony mycelia. Some colonies appear greyish-white, light cream, or dark grey (Fig. 2). The texture of the colonies varied from smooth to rough, similar to isolates found in India, with different colors and surface appearances (smooth and rough). Based on morphological observation, we presumed only four of the 12 cultivated microbial isolates to be *Pyricularia*. The colonies showed various growth types, such as downy, flat, and submerged (31, 32). The isolate had a circular shape with complete margins and the color of the colony indicated its pathogenicity; darker colors suggested more pigmentation and higher virulence (Table 3, Fig. 2).

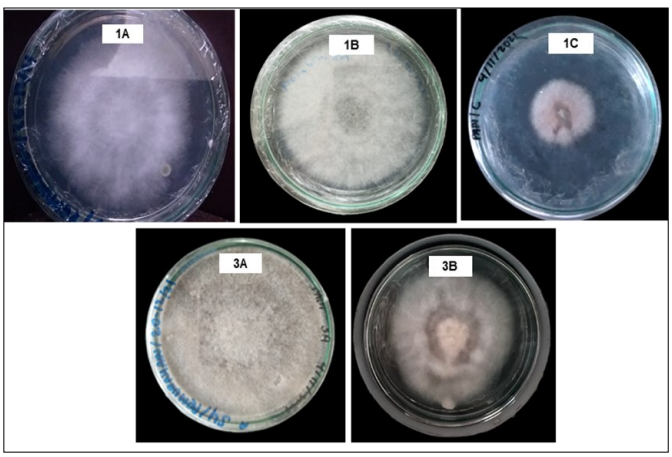

Fig. 2. Fungal isolates obtained from rice plant cultivated on PDA medium by the age of 10 day old (isolated from rice field in West Sumatra), moreover, sample 3B was not following the DNA sequencing

Molecular characterization of fungal isolates

The current study indicated that the tested isolates were associated with *P. oryzae*. BLAST query values showed that sample 1A was 98 % identical to *P. oryzae* isolate MoK19-45 and 96 % identical to MoK19-49. Samples 1B and 3A were 85 % identical with *P. oryzae* isolate MoK19-28, while sample 1C was 97 % identical with *P. caricis* isolate JAC12652 (Fig. S6).

Phylogenetic tree of blast fungus

Based on the sequences, the tested fungal isolates were separated into three common groups. Isolates 1A, *P. oryzae* isolate MoK19-49 and *P. oryzae* isolate MoK19-45 were grouped into Cluster 1, while isolate 1C, *P. caricis* isolate OP225546 and isolate JAC12652 were grouped into Cluster 2. Furthermore, isolates 1B, 3A, *P. oryzae* isolate B71 CP060330 and *P. oryzae* isolate MoK19-28 were grouped into Cluster 3. The genetic distance ranged from 0-0.4775. As presented in Fig. 3, four of the tested fungal isolates belonged to the *Pyricularia* genus. Isolates 1B and 3A had a genetic distance of 0.2389 with *P. oryzae* isolate MoK19-28 and 0.2977 with strain B71. Meanwhile, isolate 1C was designated close to *P. caricis* isolates JAC12652 and OP225546 (with genetic distances of 0.2721 and 0.3068, respectively).

Blast resistance genes (Pi) identification in rice genotypes

Out of 25 *Pi*-linked markers, 16 were polymorphic and could differentiate eight tested genotypes in four loci, including RM 1233, RM 224, RM 5926 (*Pi1*), RM 527 (*Piz5*), 195R-1 (*Pi9*), RM 6648 (*Pish*), k-6816 (*Pik*), RM 144, RM 206 (*Pikh*), Ckm-1 (*Pikm*), YL 155/

Table 3. The infection symptoms observed in eight rice genotypes after fungus *P. oryzae* inoculation (day-24)

No.	Genotype	The disease symptoms	Resistance Scale	Category
01.	Pulau Batu (PB)	The leaves turned to yellow, brown spots particularly on the leaf tips with size ±3 mm. The brown spots also found in leaf sheath with size approx. 4 cm.	5-6	Moderately resistant
02.	Cantik Manis (CM)	A few of leaves turned to yellow, no brown spots or lesion found on leaves. Small roundish pin-point size brown spots found on leaf sheath.	2-3	Resistant
03.	Bungo Sungkai (BS)	Leaf wilting and tips turned to brown and dried, small roundish brown spots (<2 mm) found on leaf sheath.	3-4	Resistant
04.	Kuriak (K)	Upper leaves turned to yellow. Brown spots and greyish lesion started on the leaf blade to margin and apex. Typical blast lesions infection approx. 30 % of the leaf area. Slightly elongated brown spots found on leaf sheath with size 0.5 – 2 cm.	7	Susceptible
05.	Mundam Putiah (MP)	Brown elongated spots with size 1-4 mm found on upper leaves. Slightly elongated brown spots found on leaf sheath with size 3-5 cm.	4-5	Moderately Resistant
06.	Inpari 48 Blas (IB)	Yellowish leaf tips covered up <4 % of upper leaf area, extended to the leaf blade with size ±3 mm. Small elongated brown spots found on the upper leaf area	3	Resistant
07.	IR64-*Sub1*	Brown small spots found on the leaf tips and margin with size ≥2 mm.	4	Moderately Resistant
08.	Inpago 9 (Ip9)	Small roundish brown spots found particularly on upper leaves and margin with size 1-2 mm (covered up <4 % of leaf area)	3	Resistant

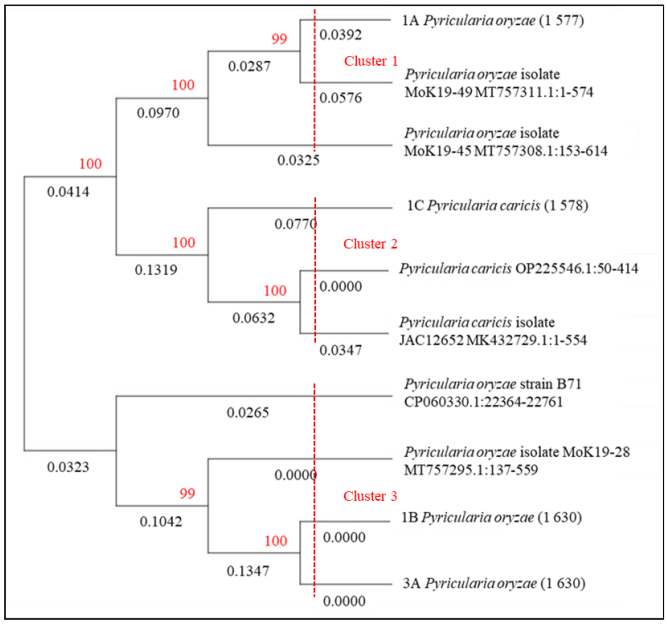

Fig. 3. Phylogenetic tree illustrates genetic relationship between fungi isolated from rice plants

YL 87 (*Pita/Pita2*), TRS26 (*Pikh*), RM 212, RM 302 (*Pi37*), 39SM (*Pi39*) and 9871.T7E2b (*Pi40*).

Based on the results, markers RM 224 and RM 302 were determined to be highly polymorphic and able to differentiate each rice genotype. Markers RM 1233, RM 5626 and RM 224 existed in locus *Pi1*. While, RM 302, along with marker RM 212, existed in locus *Pi37*. Of eight tested genotypes, only IB had the expected amplicon size of 165 bp (Fig. 4). Meanwhile, PB, CM, MP and Ip9 carried an expected amplicon size of 135 bp, while K had 110 bp and IR had 205 bp. By utilizing the DNA marker YL155/YL87, the expected amplicon size of 1042 bp was detected in the tested genotype. Genotypes PB, CM, K, IB, IR and Ip9 all displayed the expected amplicon size (Fig. 4). Table 2 summarizes the presence of Pi genes in tested rice genotypes based on DNA analysis.

Furthermore, the results indicated that marker RM 224 was able to distinguish all of the tested genotypes (Fig. 4). PCR-based analysis revealed that out of the eight examined genotypes, only K, IB, and IR exhibited a distinct expected band at

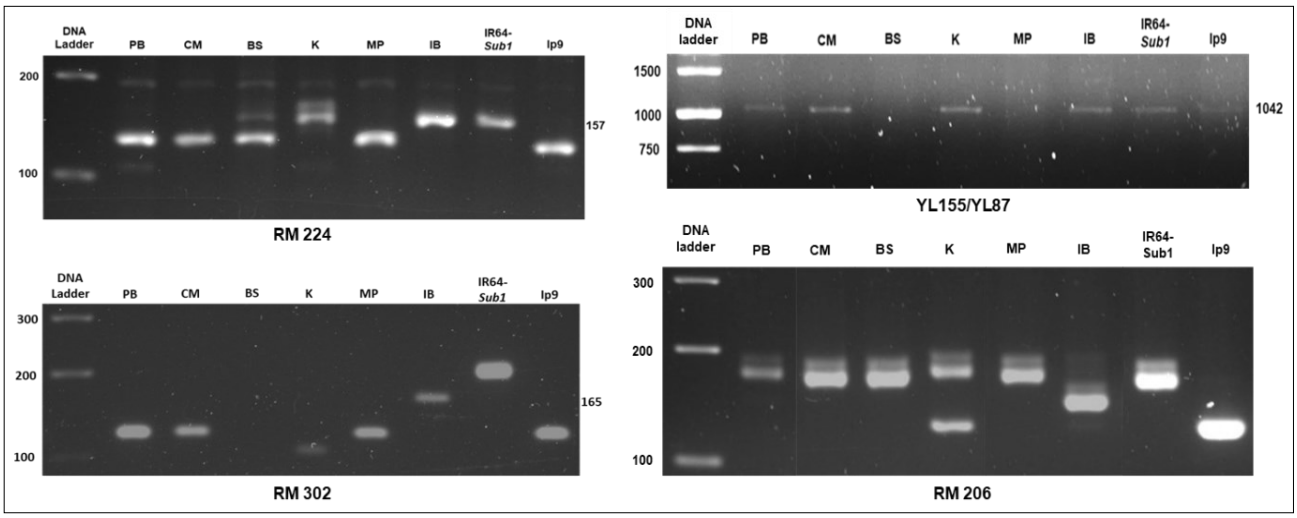

Fig. 4. PCR products of eight rice genotypes amplified using DNA marker RM 224, RM 302, YL155/YL87, and RM 206 to identify several *Pi* genes

157 bp. Meanwhile, BS exhibited a thin band shape at a similar position. In contrast, PB, CM, MP and Ip9 did not display any band at that expected amplicon size. Additionally, all examined genotypes exhibited bands at about 225 bp, except for PB and IR (which displayed bands at 235 bp), which was higher than the other genotypes. The PCR amplicons of all examined genotypes using 195R-1 showed distinct band patterns, and expected amplicon sizes of 2000 bp were only detected in CM, BS, K, IB and Ip9. Moreover, PCR products of CM, BS, IB, IR and Ip9, amplified using marker k-6816, showed the expected amplicon size at 339 bp. In comparison, K and PB exhibited lower positions at 305 bp, whereas MP occupied the topmost position at 375 bp. Utilizing marker RM 144, only CM, BS, K and MP exhibited a predicted amplicon size of 237 bp. Furthermore, with the application of RM 206, only IB exhibited an amplicon size of 147 bp.

Phenotypic blast resistance screening

After inoculation, small brown specks appeared on the plant leaf's surface and sheath before eventually enlarging into roundish, slightly elongated spots (0.1-2.0 cm length, 0.2 to 0.5 cm width) with a greyish center. Based on the lesions and affected leaf area, *P. oryzae* isolates MoK19-28 were designated as virulent. Screening on the rice genotypes showed that CM, BS, IB and Ip9 were resistant to blast fungal *P. oryzae* strain MoK19-28, with a scale of 2-4. Moreover, PB, MP and IR64-*Sub1* were identified as moderately resistant on a scale of 4-6. K was the only susceptible genotype tested in this study towards *P. oryzae* isolate MoK19-28, with a susceptibility score 7 (Table 3).

Genetic structure of rice genotypes

According to Evanno's correction, the only peak of ΔK for K=2 indicates that there are two primary rice populations (Fig. 5). The genotype classification is presented in the STRUCTURE plot (Fig. 6 A), which displays two allelic combinations of the tested rice genotypes in red and green segments. We examined two rice populations in this analysis. Population 1 (P1), shown in red, included two accessions, whereas Population 2 (P2), shown in green, included five accessions. We examined only five local rice cultivars in this analysis: PB, CM, BS, K and MP. These cultivars come from various regions in West Sumatra, with PB and MP originating from Pasaman, BS from Dharmasraya, K from Tanah Datar, and CM from Pesisir Selatan Regency. Pulau Batu (PB) and CM were placed in P1, while BS, K, and MP were placed in P2. The observed genetic relationship was not consistent with the geographical distribution in this study. For instance, PB and MP, despite hailing from the same local area, exhibited genetic differences (Fig. 6 B). This may happen due to the limited number of DNA markers used in the study and the small population size.

Discussion

Based on this study, all tested genotypes showed different symptoms and levels of severity after being infected by the blast fungus. The phenotypic screening indicated that infection symptoms experienced by plants were in line with symptoms described by IRRI (30). Some tested genotypes, such as CM, BS and Ip9, exhibited high resistance levels when infected by blast

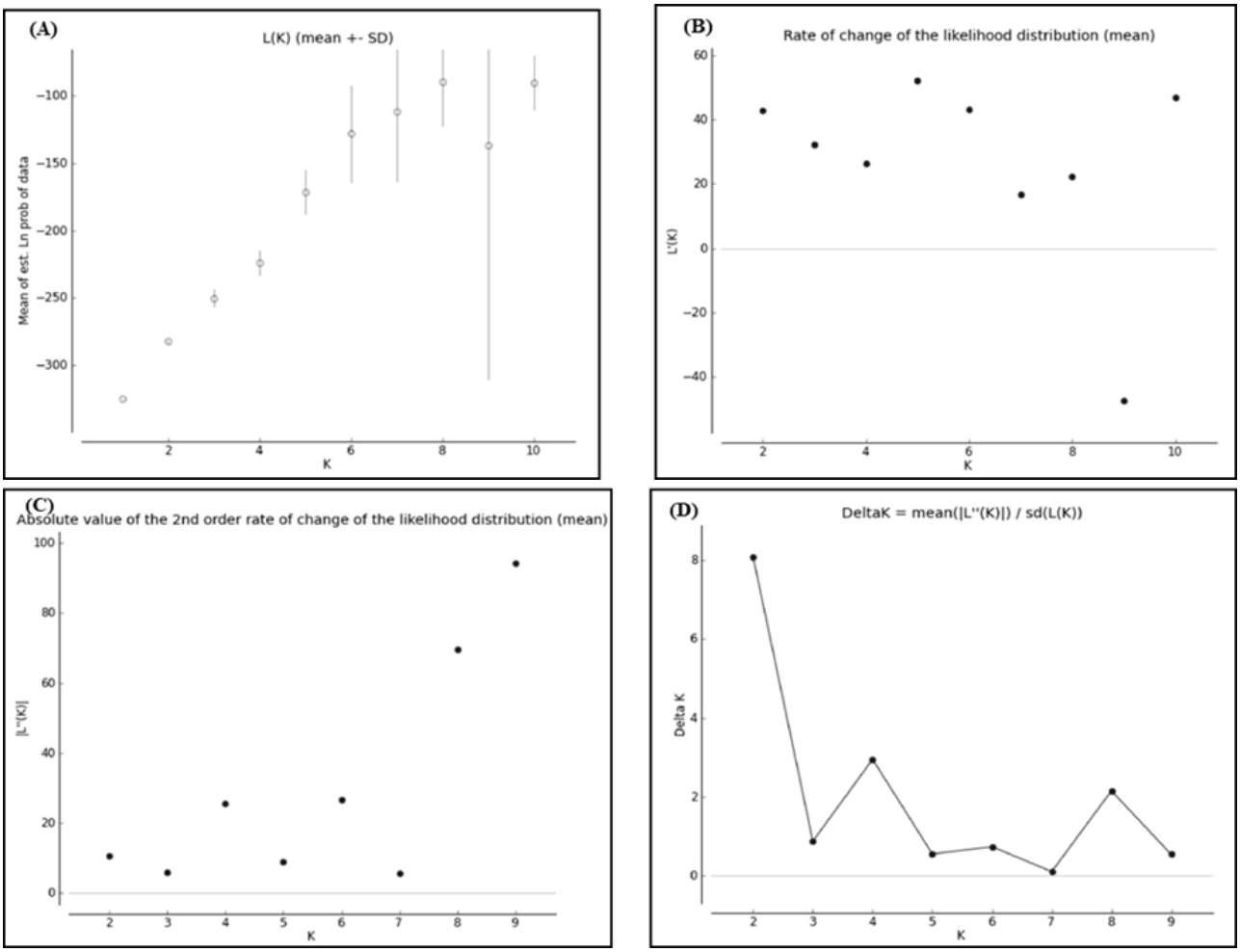

Fig. 5. Delta K-plot of Evanno's test

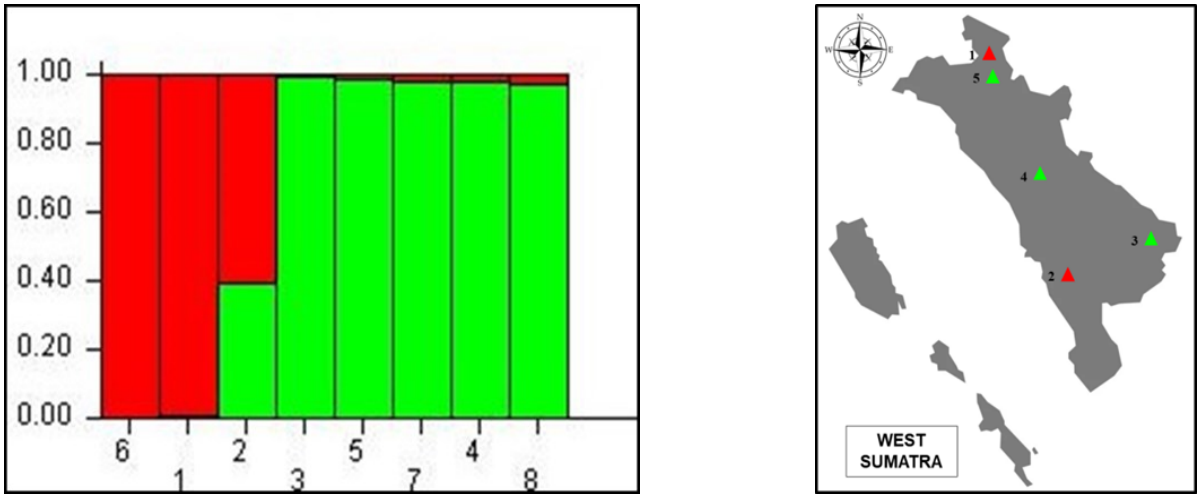

Fig. 6. (A) Structure plot of eight rice genotypes showing allelic combination and their frequencies [rice genotypes: (1) Pulau Batu/PB; (2) Cantik Manis/CM; (3) BungoSungkai/BS; (4) Kuriak/K; (5) Mundam Putiah/MP; (6) Inpari 48 Blas/IB; (7) IR64-*Sub1*/IR; (8) Inpago 9/Ip9]. (B) Distribution area of local rice cultivars originated from West Sumatra

fungus. In contrast, PB, MP and IR were identified as moderately resistant, while K was determined to be susceptible.

The variation in symptoms and severity observed during the blast fungal infection is believed to result from the genotype's ability to resist the pathogen's invasion. Testing revealed a transition in leaf pigmentation in multiple plants, changing from a pale green to either yellow or light brown. Additionally, some brown spots appeared on the upper leaf region, which may be due to the formation of dead cells or lesions. Ecological factors, age and the host plant's resistance level have an impact on rice blast symptoms. The pathogen primarily targets the plant's leaves and can induce neck or panicle blasts that cause leaf bursts during the growth or reproductive phases (33).

P. oryzae exerts a detrimental impact on the growth of rice plants. In certain plant species or genotypes, the defense system is triggered during the initial stages of infection, resulting in the absence of observable symptoms. These plants are considered resistant to pathogenic isolates or strains. In certain instances of plant-pathogen interaction, there is a temporal delay in the activation of resistance mechanisms, leading to varying levels of disease severity (34). The genotypes that facilitate unimpeded pathogen development, which leads to the manifestation of distinctive disease symptoms and the proliferation of pathogens, are termed susceptible, as this study has proven.

Based on the results, blast fungus required only a few days to infect plant cells under favourable conditions. The infection was indicated by the plant's responses after treatment, such as partial and total wilting, as well as the appearance of elongated brown spots on the upper leaf area, which then spread to other leaf areas before eventually leading to lesions. These lesions extended along the entire length of the leaf and sheath. Foliar infection begins with the attachment of three-celled conidia to the rice leaf cuticle. In Fig. S1 B fungus spores morphology under light microscope at 40x magnification can be observed. They appeared at the terminal part of the hyphae, with 3-5 conidia at each apical. These conidia grew into a dome-shaped appressorium and became mature with time. This compartment can infect rice plants when placed under appropriate conditions.

Molecular characterization analysis indicated that some tested genotypes in this study showed expected amplicon sizes. Of the 25 DNA markers used, RM 224 and RM 302 exhibited a high level of polymorphism. Along with RM 1233 and RM 5926, RM 224 has been recognized as linked to *Pi1*. On the other hand, RM 212 and RM 302 have been associated with *Pi37*, a gene proven to be responsible for a significant portion of the resistance observed in the rice cultivar against Japanese and Chinese blast isolates (9). In this study, only IB was found to have the expected amplicon size. Additionally, IB confirmed its resistance by carrying novel *Pi* genes, distinguishing it from susceptible genotypes (such as *Pi1, Pi9, Pikh* and *Pi37*). It could be argued that these R genes provide a significant contribution to the resistance against blast fungus. This is because some studies have postulated that R genes (especially the NBS-LRR group) play a crucial role in the resistance of rice plants to blast disease caused by *P. oryzae*. As previously stated, they are responsible for recognizing ligands and transmitting signals in the defense system against fungal infections (35).

Regarding the importance of R genes in plants' defense mechanism, several studies have identified and analysed *Pi37, Pish* and *Pik* as NBS-LRR (nucleotide-binding site leucine-rich repeat) class genes (36). However, it was observed that many of these genotypes that exhibit resistance have more than three *Pi* genes. For instance, the IB genotype carries multiple *Pi* genes, including *Pi1*, *Pi9*, *Pib*, *Piz-t*, *Pish*, *Pikh*/*Pi54*, *Pikm*, *Pita*/*Pita2*, *Pi37*, *Pi39* and *Pi40*. This genotype has a highly effective defense mechanism against the blast fungus *P. oryzae*. Other resistant genotypes (CM, BS, Ip9) also carry valuable and important *Pi* genes such as *Pi1*, *Pib*, *Piz-t*, *Pita*/*Pita2*, *Pi39* and *Pi40*. Some of these genes, namely *Pi1*, *Pita*, *Pi39* and *Pi40*, confer broad-spectrum resistance (BSR) (11, 37). *Pi37*, which encodes a 1290 peptide NBS-LRR, can confer complete resistance to *P. oryzae* (35, 38), while *Pish* provides partial resistance to rice blast isolates (36). Besides, *Pik* provides complete resistance against rice blast isolates (39). Other R genes, such as *Pikh*, *Pi-1*, *Pi9*, *Pi39*, *Pi40* and *Pita*, have been found to offer a broad spectrum of resistance against blast disease (11, 13).

Based on PCR-based analysis, IR64 was found to carry several blast resistance genes: *Pita*, *Pi20*, *Piks*, *Pib* and *Piz-t* (12, 39). Moreover, it also carries other genes, such as *Pia*, *Pi27-t*, *Pi29-*

t or *Pi11-t*/*Pizh*, *Pi30-t*, *Pi31-t*, or *Pita* and *Pi32-t* or *Pi12*/*Pitq6*/*Pi2-t* and a resistant locus on chromosome 9 at marker RG667 (40). This genotype has been proven to confer resistance to blast disease under irrigated conditions in Thailand (41). Nevertheless, current investigation has demonstrated that the IR64-*Sub1* exhibits only a modest level of tolerance to the *P. oryzae* isolate MoK19-28.

In this study, genetic relationships did not correspond with geographical distribution. For example, despite coming from the same local area, PB and MP were genetically different. This may occur due to the small population size and limited number of DNA markers used in the study. Due to stochastic sampling error (i.e., genetic drift), small populations tend to lose genetic diversity more rapidly than large populations. Nonetheless, some variants of a gene may be lost as a result of random chance.

Conclusion

This study successfully discovered three blast fungus isolates: *P. oryzae* MoK19-28, *P. oryzae* MoK19-45 and *P. oryzae* MoK19-49. The *P. oryzae* isolate MoK19-28 was designated as a virulent isolate that can cause a severity level of 7 in genotype K. Four of the screened genotypes; CM, BS, IB and Ip9 were resistant to leaf blast (*P. oryzae* isolate MoK19-28) based on blast-resistance testing. Additionally, genotypes PB, MP and IR showed moderate resistance. Genetic analysis revealed that the resistant genotype IB contained the most *Pi* genes, including *Pi1*, *Pi9*, *Pikh* and *Pi37*, distinguishing it apart from the susceptible genotypes. It can be argued that these R genes provide a significant contribution to the resistance against the blast fungus. Most of the *Pi* genes identified in this study were NBS-LRR R genes that are responsible for ligand recognition and signal transduction in defense mechanisms during fungal infections. It is hypothesized that these R genes contribute to the resistance of rice plants to blast disease caused by *P. oryzae*.

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Authors' contributions

SDP carried out the phenotypic test and molecular genetic studies, statistical analysis and drafted the manuscript. NAAS participated in the design of the study, performed the statistical analysis and drafted the manuscript. NLS and JJ conceived of the study and participated in its design and coordination. NMY and SMN participated in data analyses. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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