
Determination of race virulence of rice-blast fungus of *Pyricularia oryzae* in five districts of Bengkulu Province, Indonesia

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Abstract The attack of blast disease caused by *Pyricularia oryzae* on lowland rice was quite extensive in Bengkulu Province. The previous monitoring showed that the blast disease in rice caused by *P. oryzae* was quite extensive, but the disease transmission was not well documented. Information on the distribution of *P. oryzae* races is needed to predict which rice variety is suitable to release at a specific location. Identification and isolation of resistant genes from hosts and pathogens with avirulence genes are currently needed to deepen the molecular mechanisms involved in pathogen-host interactions and to develop strategies for introgression of resistant genes into the high-yielding varieties. The result obtained was 15 isolates of *P. oryzae* in 5 districts in Bengkulu. Based on the reactions of *P. oryzae* isolates in 7 varieties, 7 races were found with different numbers at each location. Of the total 7 races obtained, race 001 was the dominant race found in North Bengkulu, Pajar Bulan Village, Karang Anyar, Semidang Alas Maras, Cawang Lama, and Air Putih. Race 003 was found in Talang Benih and Kembang Mumpo, race 013 was found in Sendawar village, race 043 was found in Rawa Makmur, and races 333, 341, and 373 were found in Central Bengkulu, Cawang Lama 2, and South Bengkulu. *P. oryzae* contains one or more unstable virulence genes. Frequent spontaneous mutations may affect its virulence, which may cause a high genetic diversity of blast pathogens. The status of the *P. oryzae* races might determine the location-specificity of rice cultivars. Planting resistant varieties is an appropriate strategy to control blast disease in endemic areas of *P. oryzae* races.

Keywords: Local isolates, Blast disease, *P. oryzae*, Virulence, Race

Introduction

Bengkulu Province is located in the southern part of Sumatra Island of Indonesia, which has a fairly wide landscape of lowland and upland rice. The previous monitoring showed that blast disease on rice caused by *Pyricularia oryzae* was quite extensive, but the disease transmission was not well documented. Sudir *et al.* (2014) reported that the blast disease had developed on upland to lowland rice, including the variety of IR64. The high genetic diversity

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of *P. oryzae* races, and changes in the virulence are the main factors that cause the blast disease resistance of rice varieties to easily break (Mulyaningsih *et al.*, 2016; Sheoran *et al.*, 2021).

Blast disease, caused by the fungus *Pyricularia oryzae* (Cooke) Sacc. (synonym *Pyricularia grisea* Cav., anamorph *Magnaporthe grisea* (Hebert) Barr.), is an important disease in rice because the level of damage is quite high (Wang *et al.*, 2014; Sudir *et al.*, 2014; Hossain *et al.*, 2017). Fungus blast has a high level of genetic diversity and consists of many races that are widely distributed in various geographical locations and seasons (TaHERi and Irannejad, 2013; Shahriar *et al.*, 2020). Some factors that cause diversity in blast populations were ability to migrate, spontaneous mutation, sexual hybridization, parasexuality, and heterokaryosis (Wang and Valent, 2017; Sheoran *et al.*, 2021).

Another factor that changes the resistance of rice varieties is the difference in the composition of the *P. oryzae* races in one area. This characteristic makes it difficult to develop control strategies against blast disease.

Blast control is generally carried out by improving cultivation techniques, diversifying rice varieties, planting blast-resistant varieties, and using fungicides (Chakraborty *et al.*, 2020; Mutiga *et al.*, 2021). Planting blast-resistant varieties is the most effective, economical, and easy to do, but it is limited by time and place. Planting the resistant varieties must be adjusted to the composition of the *P. oryzae* race in an area or specific location (TaHERi and Irannejad, 2014; Sudir *et al.*, 2014). Therefore, planting the resistant varieties must be supported by data on the composition of the *P. oryzae* race in an area (Sudir *et al.*, 2014; Hu *et al.*, 2002; Kawasaki-Tanaka *et al.*, 2016; Liu *et al.*, 2017). Thus, it is necessary to monitor *P. oryzae* races in each rice agroecosystem, especially the endemic areas, to prevent blast disease (TaHERi and Irannejad, 2014; Santoso *et al.*, 2019; Zhang *et al.*, 2022).

This control strategy must adjust the distribution and the dominance of *P. oryzae* races found in the area (Sudir *et al.*, 2014; Kawasaki-Tanaka *et al.*, 2016). A previous study in Lampung, Indonesia, during the 2004-2005 rainy season reported that blast races in blast-endemic areas were successfully identified (Utami *et al.*, 2011). The results indicated that races 001, 033, and 173 were always found in the tillering and flowering stages as leaf blasts, as well as in the near-harvest stage as a neck panicle blast. In general, two blast attacks are known, namely leaf blast, which attacks rice plants on the leaves, especially in the vegetative phase, and panicle neck blast, which attacks at the beginning of flowering (Kalia and Rathour, 2019; Shahriar *et al.*, 2020).

Identification and isolation of resistant genes from hosts and pathogens with avirulence genes are currently needed to deepen the molecular mechanisms involved in pathogen-host interactions and to develop strategies for introgression

of resistant genes into the high-yielding varieties (Mbinda and Masaki, 2021; Herawati *et al.*, 2022). Molecular markers are widely used to characterize gene bank collections that contain untapped resources of different alleles (Herawati *et al.*, 2017) that are essential for identifying broad-spectrum blast-resistant genes to be effective in addressing dynamic blast races (Jiang *et al.*, 2019; Sheoran *et al.*, 2021; Herawati *et al.*, 2022). The use of molecular markers is very helpful for detecting several blast-resistant genes that have the potential to be durable resistance to obtain pyramiding genes that can overcome multi-race and very dynamic blast pathogens (Xiao *et al.*, 2016; Orasen *et al.*, 2020).

Several blast-resistant genes have been identified, and monogenic lines carrying blast-resistant genes have been developed into different varieties (Stam *et al.*, 2014; Wiesner-Hanks and Nelson, 2016; Herawati *et al.*, 2022). The presence of the main R gene in plants prevents infection by the *P. oryzae* race harboring the appropriate avirulence (Avr) gene; however, R-mediated plant resistance usually loses over time, as the pathogen can develop new races through gene recombination or mutation of avirulence genes (Starnes *et al.*, 2012; Wang and Valent, 2017). Varieties that have a single gene generally only overcome virulent races that appear. Previous research revealed that the presence of several major resistance genes can provide broad-spectrum resistance for a long time (Xiao *et al.*, 2016; Li *et al.*, 2020; Herawati *et al.*, 2022).

The presence of several different resistant genes in the same plant prevents infection from multiple pathogenic races, thereby preventing the pathogen from evolving by preventing recombination between different pathogenic races (Nickolas *et al.*, 2018; Herawati *et al.*, 2022). This approach allows for a wider and longer-lasting tolerance to blast disease. Previous researchers have successfully carried out a pyramid program to achieve longer-lasting resilience (Xiao *et al.*, 2016; Orasen *et al.*, 2020). The objective of this study was to identify and determine the race and the virulence of *P. oryzae* in five districts of Bengkulu Province, Indonesia, and to develop a strategy for controlling blast disease.

Materials and methods

Field survey for blast disease

Samples of blast-infected plants were determined by survey and collected from the rice centers in 6 districts of Bengkulu, namely Bengkulu City (1), North Bengkulu District (2), Central Bengkulu District (3), South Bengkulu District (4), Rejang Lebong District (5), and Seluma District (6) (Figure 1). Sampling plants with blast disease symptoms focused on rice varieties that were always

grown by the local farmers. Plant samples collected were parts of rice showing disease in the vegetative and generative stages.

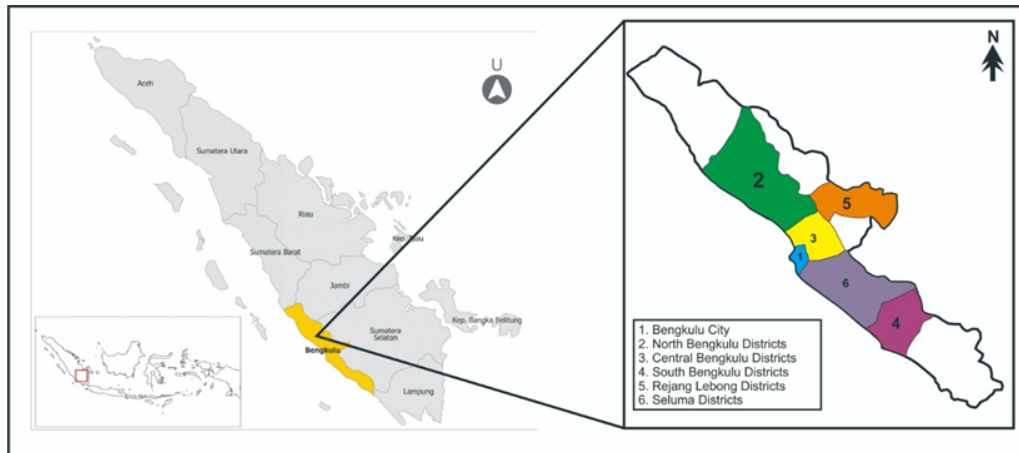


Figure 1. Survey's locations in 5 districts of Bengkulu Province: Bengkulu City (1), North Bengkulu District (2), Center Bengkulu District (3), South Bengkulu District (4), Rejang Lebong District (5), Seluma District (6)

Identification of Pyricularia oryzae

Identification of the fungus *P. oryzae* was carried out in the Plant Protection Laboratory, University of Bengkulu. Leaves and collars, stem nodes, and neck panicles of infected plants were taken for laboratory analysis. Isolation of the fungus using the filter paper (blotter test) and agar methods, modified from the method of Hayashi and Fukuta (2009). The leaves were cut to a size of 1–2 cm², while the stem nodes and neck panicles were cut to a size of 3–5 cm². The cut tissues were sterilized by immersing them in 1% NaOCl for 3 minutes and washing twice with sterile distilled water. Three pieces of filter paper were moistened in a sterile petri dish, then the cut leaves were placed on the filter paper and incubated at room temperature with irradiation for 12 hours in the dark and 12 hours in light with a NUV lamp for 5–7 days. Emerged conidia from plant tissue were observed with a binocular microscope. The growing conidia were incubated on a PDA medium at room temperature for 7–10 days. The mycelium grown on plant tissue was purified by subculturing it in the dishes containing PDA medium and incubating it at 25°C for 7–10 days. The fungal culture was maintained in the dark for 12 hours to induce sporulation, then the conidia were observed using a microscope (Srivastava *et al.* 2014). *P. oryzae* was identified by the presence of a club-like conidium that is gray to hyaline in color and

generally has 2 septa, but sometimes 1 or 3 septa are found (Bonman *et al.*, 1986). The culture identified as *P. oryzae* was purified and stored as a collection of cultures. This fungus is propagated to be used as an inoculum source for testing the fungus race.

Determination of race *P. oryzae* virulence using differential rice varieties

Determination of the *P. oryzae* race followed the method of the Rice Research Center (Prabawa *et al.*, 2015). The test was done on seven differential varieties of rice, namely Asahan, Cisokan, IR64, Krueng Aceh, Cisadane, Cisanggarung, and Kencana Bali. The preparation of the inoculum followed the methods of Hayashi and Fukuta (2009). The surface of the *P. oryzae* colony of each isolate was poured with 10 mL of sterile distilled water containing 0.02% Tween-20, then rubbed using brush number 10 to obtain the conidium suspension. Inoculation was done 18–21 days after planting by spraying a conidium suspension of 1×10^6 conidia mL^{-1} . Each treatment was repeated three times. Observations were done 7 days after inoculation based on the standard evaluation system for the rice observation scale (IRRI, 2013) (Table 1).

The race and virulence of *P. oryzae* were determined using the method developed by Mogi *et al.*, 1991. After obtaining the data on the resistant reaction of different varieties to *P. oryzae*, race identification can be carried out based on the sum of the different variety code numbers indicating a susceptible reaction (S). In this case, for example, race number 001 showed a resistant reaction (R) to all different varieties but is susceptible (S) to the Kencana Bali variety (001), so it is determined as race 001.

Data analysis

The following formula by IRRI (2013) was used to determine the severity of the disease:

$$DS = \frac{\sum_{i=0}^n (n_i x v_i)}{N \times Z} \times 100\% \quad (1)$$

where DS is disease severity; v is the score according to symptom criteria in the i-th family; n_i is the number of families attacked in the i-th score; N is the total clumps observed; and Z is the highest score.

Table 1. The scale of blast disease symptoms for field assessment based on SES IRRI

Scale	Symptoms
0	No lesions were observed.
1	Tiny brown specks of pin-point size or larger brown specks without
2	sporulating centers
3	Small, roundish to slightly elongated necrotic gray spots with a distinct brown margin, about 1-2 mm in diameter.
4	The lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves.
5	Typical susceptible blast lesions are 3 mm or longer, infecting less
6	than 4% of the leaf area.
7	Typical blast lesions infect 4–10% of the leaf area.
8	Infection of 11-25% of the leaf area by typical blast lesions
9	Infection of typical blast lesions: 26–50% of the leaf area
	Typical blast lesions: many leaves are dead, accounting for 51–75 percent of the leaf area.
	More than 75% of the leaf area has been damaged.

Results

Field survey for blast disease

Samples of plants with blast disease symptoms were collected from Bengkulu City in Rawa Makmur sub-district, North Bengkulu District in Sumber Agung village, Central Bengkulu District in Lubuk Sini village, South Bengkulu District in Seginim and Pajar Bulan villages, Seluma District in Semidang Alas Maras village, Sendawar, Karang Anyar, and Kembang Mumpo, and Rejang Lebong District in Talang Benih, Cawang Lama, and Air Putih villages (Table 2). Leaf blast diseases were observed on IR 64, Inpari, Ciherang, Koning, Koreng, and Situbagendit varieties. No plants were affected by the panicle neck blast. Plant samples were put in plastic and labeled for the location, date of collection, and variety (Figure 2) for further isolation and identification in the laboratory.

Table 2. Sample locations of blast disease in Bengkulu Province, Indonesia

Location	Village	Coordinate	Number of isolates	Varieties	Isolate Code
South Bengkulu District	Seginim	4°26'02.52"S 103°00'58.35"E	1	IR64, Ciherang	BS
	Pajar Bulan	4°26'40.48"S 103°00'30.00"E	1	IR64	PB
North Bengkulu District	Sumber Agung	3°28'04.47"S 102°14'25.75"E	1	Ciherang	BU

Central Bengkulu District	Lubuksini	3°41'32.97"S 102°28'51.80"E	1	Ciugeulis	BT
Bengkulu City	Rawa Makmur	3°46'43.11"S 102°16'34.51"E	1	IR64, Ciherang Kuning	RM SAM
Seluma District	Semidang Alas Maras	4°18'27.27"S 102°45'42.32"E	2		
	Sendawar	4°14'02.43"S 102°46'36.22"E	1	IR64, Ciherang	SEND
	Karang Anyer	4°18'14.45"S 102°47'17.62"E	1	IR64, Ciherang	KA
	Kembang Mumpo	4°18'25.42"S 102°45'23.80"E	1	IR64, Ciherang	KM
Rejang Lebong District	Talang Benih	3°28'27.21"S 102°30'35.02"E	1	Situbagendit	TB
	Cawang Lama	3°22'55.11"S 102°34'53.19"E	3	Koreng	CL
	Air Putih	3°29'06.60"S 102°31'21.48"E	1	IR64	AP



Figure 2. Observation of the blast disease symptoms in the rice field in Bengkulu Province

*Identification of the fungus *Pricularia oryzae**

P. oryzae caused leaf blast was obtained from 15 isolates in Bengkulu Province, as shown in Table 3. In general, the fungi that cause leaf blasts were found in rice varieties of IR-64, Ciherang, Situbagendit, and Koreng. Leaf blast diseases were found on varieties of IR64 and Ciherang in almost all areas of Bengkulu Province, while the leaf blast disease on local varieties such as Kuning and Koreng was found in South Bengkulu and Rejang Lebong Districts. The survey did not identify the panicle blast disease. The lowland rice varieties in Bengkulu Province were susceptible to leaf blast disease. The pure isolates of *P. oryzae* for each isolate are shown in Figure 3.

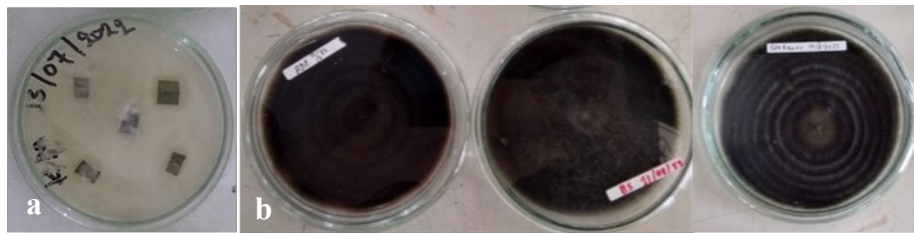


Figure 3. Identification of isolate blast: (a) the growth of isolate in Petri dish after purification; (b) pure culture of blast

Assessment of isolates on differential rice varieties

Symptoms of blast disease caused by inoculation of the fungus on different varieties were divided into 2 categories, namely resistant (R) with a score of 0–4 and susceptible (S) with a score of 5–9 (Figure 4d). Symptoms of blast attack on resistant varieties were small brown lesions that did not develop significantly (Figure 4a and b), while on susceptible varieties, the lesions were characterized by white to grey sporulation center with brown edges and tapering ends (Figure 4c). Lesions on susceptible varieties coalesced and covered the entire leaf surface (Figure 4d); under humid conditions, the leaves will eventually rot and die. Symptoms of blast disease begin with brown needle-like spots. If the rice variety is susceptible, the spots may then develop into an oval and sometimes a rhombic shape. The center of the spot is white-grey with brown edges. The size and color of the spots depend on the degree of susceptibility to the variety and the environmental conditions.

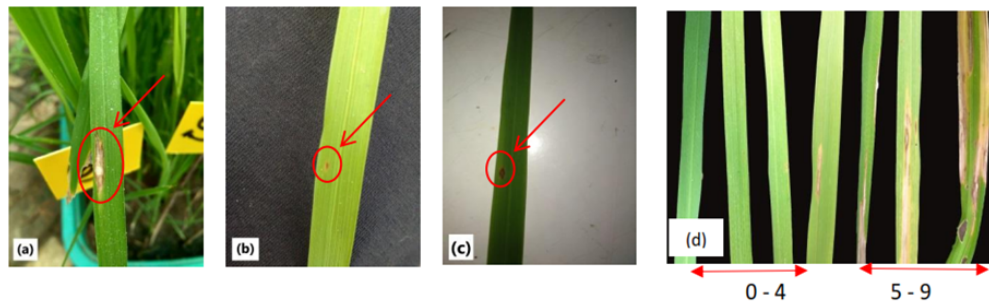


Figure 4. Leaf blast symptoms (a); early symptoms of blast disease (b); blast disease after sporulation (c); blast resistance score (d)

Table 3. Reaction of different rice varieties to 15 isolates of *P. oryzae*

Isolate	Race	Reaction of differential rice varieties*						
		Asahan (200)	Cisokan (100)	IR64 (040)	K. Aceh (020)	Cisadane (010)	Cisangg arung (002)	K. Bali (001)
BT	333	S	R	R	S	S	S	S
BU	001	R	R	R	R	R	R	S
BS	373	S	S	S	S	S	S	S
RM	043	R	R	S	R	R	S	S
PB	001	R	R	R	R	R	R	S
TB2	003	R	R	R	R	R	S	S
CL2	341	S	S	S	R	R	R	S
KA	001	R	R	R	R	R	R	S
KM	003	R	R	R	R	R	S	S
SAM1	001	R	R	R	R	R	R	S
CL1	001	R	R	R	R	R	R	S
CL3	001	R	R	R	R	R	R	S
AP	001	R	R	R	R	R	R	S
SAM 1	001	R	R	R	R	R	R	S
SDR	013	R	R	R	R	S	S	S

*Scoring base on IRRI (2013); Resistant (R) with a score of 0–4 and susceptible (S) with a score of 5–9

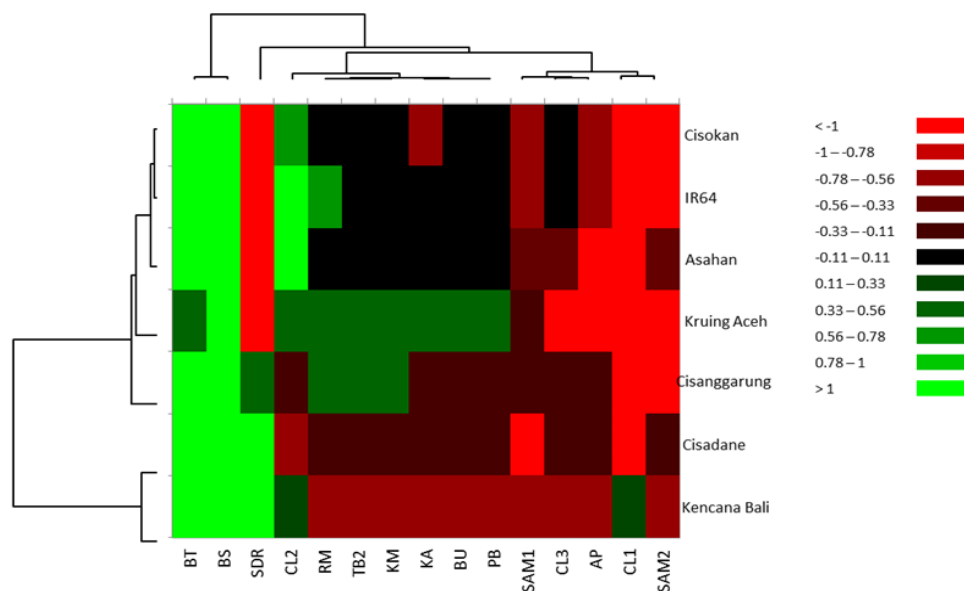


Figure 5. Heat map to show the virulence of isolates based on the score of SES IRRI (Standard Evaluation System International Rice Research Institute) on different varieties, High values are indicated in green and low values in red

Based on the reactions of *P. oryzae* isolates in 7 varieties, 7 races were found with different numbers at each location (Table 3). Of the total 7 races obtained, race 001 was the dominant race found in North Bengkulu, Pajar Bulan Village, Karang Anyar, Semidang Alas Maras, Cawang Lama, and Air Putih. Race 003 was found in Talang Benih and Kembang Mumpo, race 013 was found in Sendawar village, race 043 was found in Rawa Makmur, and races 333, 341, and 373 were found in Central Bengkulu, Cawang Lama 2, and South Bengkulu (Table 3). Based on the differential variety test, race 373 is the most virulent race because it can infect all varieties (most R codes). The higher the race code, the more virulent a race is. Race 001 has the lowest virulence because it is only capable of infecting the most sensitive variety, var Kencana Bali. A heat map to show the virulence of isolates based on the score of SES IRRI (Standard Evaluation System International Rice Research Institute) on differential varieties. High values are indicated in green, and low values are in red (Figure 5).

Clustering analysis based on isolate virulence resulted in 3 groups (Figure 6). Group 1 was isolated from Central Bengkulu, South Bengkulu, and Sendawar; group 2 was isolated from North Bengkulu, Rawa Makmur, Pajar Bulan, Talang Benih, Cawang Lama, Karang Anyar, Kembang Mumpo, Semidang Alas Maras,

Cawang Lama 3, and Air Putih, while groups of 3 were isolated from Cawang Lama 1 and Semidang Alas Maras 2.

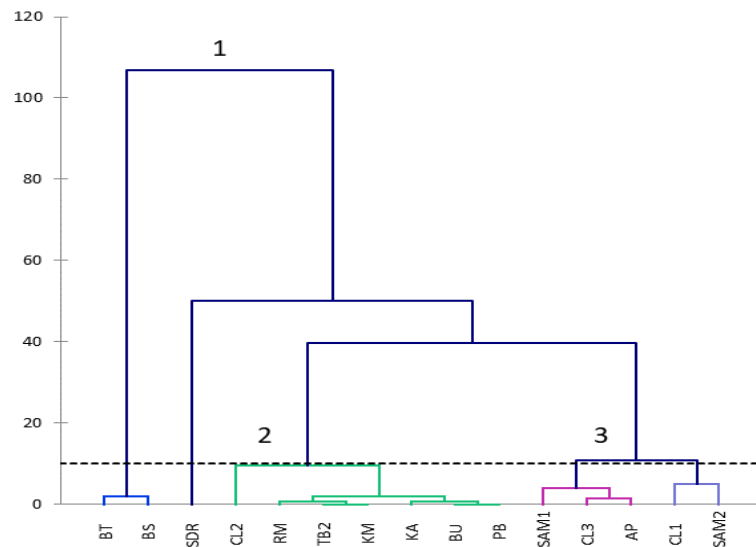


Figure 6. Clustering of blast isolates based on their virulence into differential varieties

Discussion

Evaluation of *P. oryzae* races (phenotypes) has been widely investigated in various parts of the world. In Indonesia, phenotypic testing is generally carried out by monitoring the development of blast pathogen populations in the field. The blast disease severity survey conducted on lowland rice in Bengkulu Province showed varied data. The difference in the severity of blast disease is influenced by the resistance level of each variety and the age of the plant. In some cases, susceptible varieties showed resistant responses because of the age of the plant. The older the plant, the more severe the disease because the number of leaves and rice tillers increases, causing more micro humidity around the rice (Akhsan and Palupi, 2015; Herawati *et al.*, 2021).

We conducted a blast disease severity survey in 5 districts and the city of Bengkulu, which was based on the virulence reaction to a set of different varieties (Mogi *et al.*, 1991). We found 7 races of fungi with varying numbers at each location. From a total of 7 races obtained, race 001 was the dominant race, and races 333, 341, and 373 were the most virulent races found in Central Bengkulu, Cawang Lama, and South Bengkulu, respectively. Each race has different

virulence traits (Valent and Chumley, 1994). The dominance of the *P. oryzae* race in an area with other different areas allows rice varieties to be resistant in one area but susceptible in others. Information on the distribution of *P. oryzae* races is needed to predict the suitable rice variety to be released at a specific location. Varieties of Cisokan and Kruing Aceh were the most resistant (R) to 13 of the 15 isolates tested, while varieties of IR 64, Asahan, and Cisadane were resistant to 12 isolates, var Cisanggarung was resistant to 9 isolates, and var Kencana Bali showed the lowest resistance spectrum. These results are consistent with previous studies reported by Lestari *et al.*, 2014. The Kencana Bali variety was always used as a susceptible check in blast testing in Indonesia (Mogi *et al.*, 1991).

Clustering analysis based on isolate virulence resulted in 3 groups (Figure 6). Allegedly, the grouping of blast isolates is influenced not only by the geography of the origin of the isolates but also by the time of isolation. The blast isolates tested had a wide spectrum of virulence against differential varieties. Isolates that were proven to be races 373, 341, and 333 were categorized as the most virulent because almost all of them were susceptible to all different varieties, while races 043 and 013 belonged to the moderately virulent group, and races 003-001 were categorized as the least virulent because almost all varieties are resistant to different varieties except the variety Kencana Bali. According to Utami *et al.* (2011), race 001 represents the blast race belonging to group 1, which has a very low virulence but showed a wide distribution and can survive for a long time in the field. Race 003 represents the blast race belonging to group 2, which has a very diverse virulence level and can adapt to the selection pressures, and race 173 represents the blast race belonging to group 3, which has a high virulence but does not last long in the field. Classification of blast isolates based on the virulence spectrum is useful for evaluating the resistance of rice cultivars to blast disease.

Race 001 is the most dominant in Indonesia (Mogi *et al.*, 1991; Santoso *et al.*, 2019). The dominant blast pathogen race in an area can change due to changes in genetic composition and high mutation rates in blast fungi (Xu *et al.*, 2021). Changes in race can be influenced by differences in rice cultivars planted in an area (Kawasaki-Tanaka *et al.*, 2016). Race dominance in the same area can change over time depending on the growing season and changing ecosystems (Khan *et al.*, 2014).

Knowledge of the race status of *P. oryzae* in an area will support rice cultivar regionalization programs to determine site-specific rice cultivars. In addition, blast disease control strategies in endemic areas by planting resistant varieties will be more effective if supported by information on the distribution of *P. oryzae* races. The identification of the *P. oryzae* race in the Bengkulu region

aims to establish location-specific resistant varieties according to the distribution of the composition of the *P. oryzae* race in the area. Wang *et al.* (2017) reported that the resistance gene carried by a variety to control certain blast races in an area cannot necessarily be developed in other areas.

Race *P. oryzae* with a high level of virulence in South Bengkulu and Rejang Lebong requires control as early as possible. Yulianto (2017) states that the main difficulty in controlling blast disease is using resistant varieties if the variety has only a single resistance gene that is only resistant to certain blast races. If planted continuously, these varieties will become susceptible and easily infected with new pathogenic races in the area. The intensity of blast disease transmission depends on the varieties used, blast pathogens, and environmental factors. Considering this, the blast disease control that can be done is through the diversification approach of high-yielding resistant varieties in a certain stretch. The use of several varieties in a field is reported to inhibit changes in pathogen virulence, thereby increasing yield stability. This is shown by the research of Castilla *et al.* (2010), who proved the use of several varieties in a field in Lampung, Indonesia, can reduce the intensity of blast disease transmission and increase grain yields. The decrease in blast disease intensity and increase in yield depended on the composition of the resistant varieties used.

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Conflicts of interest

The authors declare no conflict of interest.

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