

Resistance of Local Acehnese Rice Lines to *Xanthomonas Oryzae* pv. *Oryzae*, the Causal Agent of Bacterial Leaf Blight Disease

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ABSTRAK

Bacterial leaf blight, yang disebabkan oleh bakteri *Xanthomonas oryzae* pv. *oryzae*, merupakan masalah utama bagi produksi padi di Indonesia. Salah satu cara untuk mengendalikan penyakit ini adalah dengan menggunakan varietas padi yang tahan. Penelitian ini bertujuan untuk memperoleh galur padi lokal Aceh, memperbaikinya melalui pemuliaan inbred, dan menggunakan iradiasi sinar gamma untuk mengendalikan bacterial leaf blight. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) non-faktorial dengan sembilan galur padi lokal Aceh, yaitu Tinggong (T.35.7.5.7.1), Tinggong (T.35.7.5.1.1), Sambay Simeulue (US1), Sambay Simeulue (US2), Sigupai (UM69), Sigupai (UM18), Sikuneng Bireuen (UB5), Sikuneng Bireuen (UB9), dan varietas Ciherang sebagai pembanding, dengan tiga ulangan. Masa inkubasi dan kejadian penyakit tidak berbeda secara signifikan antara perlakuan, berkisar antara 4,33 hingga 5,66 hari dan dari 39,28 hingga 57,03%, masing-masing. Tingkat keparahan penyakit tertinggi diamati pada varietas padi Sigupai (UM69) (47,37%), dan terendah pada Sambay Simeulue (US2) (26,25%). Hasil ini menunjukkan bahwa galur padi lokal Aceh masuk ke dalam kategori sedang tahan, yaitu Sambay Simeulue (US2), Tinggong (T.35.7.5.1.1) dan Sambay Simeulue (US1). Sebaliknya, kategori sedang rentan adalah Sikuneng Bireuen (UB9), Sikuneng Bireuen (UB5), varietas Ciherang (pembanding), Tinggong (T.35.7.5.7.1), Sigupai (UM18) dan Sigupai (UM69).

Kata kunci: Ketahanan, *Xanthomonas*, varietas lokal, hawar daun, tahan

ABSTRACT

Bacterial leaf blight, caused by the bacterium *Xanthomonas oryzae* pv. *oryzae*, is a major problem for rice production in Indonesia. One way to control this disease is to use rice varieties with resistance. The study aims to obtain local Acehnese rice lines, improve them through inbred breeding, and use gamma-ray irradiation to control bacterial leaf blight. The study used a non-factorial Complete Random Design (CRD) with nine local Acehnese rice lines, namely Tinggong (T.35.7.5.7.1), Tinggong (T.35.7.5.1.1), Sambay Simeulue (US1), Sambay Simeulue (US2), Sigupai (UM69), Sigupai (UM18), Sikuneng Bireuen (UB5), Sikuneng Bireuen (UB9), and Ciherang varieties as comparisons, with three replicates. The incubation period and disease incidence were not significantly different between treatments, ranging from 4.33 to 5.66 days and from 39.28 to 57.03%, respectively. The highest disease severity was observed in the Sigupai rice strain (UM69) (47.37%), and the lowest in Sambay Simeulue (US2) (26.25%). The results indicate that the Aceh local rice lines fall into the moderately resistant category, namely Sambay Simeulue (US2), Tinggong (T.35.7.5.1.1) and Sambay Simeulue (US1). In contrast, the moderately susceptible categories are Sikuneng Bireuen (UB9), Sikuneng Bireuen (UB5), Ciherang variety (comparator), Tinggong (T.35.7.5.7.1), Sigupai (UM18) and Sigupai (UM69).

Keywords: Resistance, *Xanthomonas*, local varieties, leaf blight, resistant

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of Indonesia's staple food crops. The demand for rice continues to rise each year as the population grows. According to BPS data, rice production in Aceh Province in 2022

was 1.51 million tonnes of GKG, down from 1.63 million tonnes of GKG in 2021. One of the factors behind low rice production is pathogen attack (Handayani et al. 2023).

Bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a pathogen of bacterial leaf blight (BLB) and is one of the main diseases of rice plants. This disease can reduce rice production by 15-80%, depending on the stage of the rice plant (Lestari et al. 2023). According to Yuliani and Sudir (2017), the symptoms in the vegetative phase are called kresak, characterised by a change in the colour of the leaves to grey at the edges and tips, after which the rice plants wilt and die. In the generative phase, the symptoms are called blight. This disease interferes with photosynthesis and reduces the production of assimilates, causing hollow grain.

Currently, various strategies have been used to control BLB disease, including the use of synthetic pesticides. Excessive use of synthetic bactericides can cause bacterial resistance, health issues, and environmental pollution (Nurawan et al. 2021). Therefore, using superior varieties resistant to BLB disease is a way to increase rice production (Yuriyah et al. 2013). Aceh's local rice, including Sigupai, Tinggong, Sikuneng, and Sambay Simeulue, has been genetically improved through breeding, hybridisation, and mutation and has the potential for agronomic and production characteristics that exceed those of their elders (Harta and Mahyaruddin. 2022; Efendi et al. 2023 and Zakaria et al. 2021).

Therefore, research is required to test the resistance of local Acehnese rice lines, whose genetic characteristics have changed through cross-breeding and gamma radiation, to bacterial leaf blight in a screen house. The purpose of this research was to determine the level of resistance of several strains of local Acehnese rice plants to bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*.

METHODS

Location Research and preparation of rice seeds

This research was conducted in a semi-screen house in Baet Village, Aceh Besar Regency (5°35'19"N 95°21'48"E), and in the Laboratory of Plant Protection, Faculty of Agriculture, Syiah Kuala University, from December 2023 to May 2024. The materials used included BC4F3-derived rice seeds from the crossing of Tinggong and IRBB27, consisting of two lines collected from the Laboratory of Genetika dan Pemuliaan Tanaman, Faculty of Agriculture, Syiah Kuala University; Sigupai rice lines (gamma-ray irradiated at 250 Gy); Sambay Simeulue rice (gamma-ray irradiated at 250 Gy); Sikuneng Bireuen (crosses of Sikuneng and IRBB27) from the Aceh Rice Research Institute (ARRI), Banda Aceh, Eastern Sector; and Ciherang Varieties as a comparison.

Experimental design

This research employed a non-factorial Complete Random Design (CRD) with 9 treatments and 3 replicates. The treatment arrangement was G1 = Tinggong (T.35.7.5.7.1), G2 = Tinggong (T.35.7.5.1.1), G3 = Sambay Simeulue (US1), G4 = Sambay Simeulue (US2), G5 = Sigupai (UM69), G6 = Sigupai (UM18), G7 = Sikuneng Bireuen (UB5), G8 = Sikuneng Bireuen (UB9), and V = Ciherang.

Preparation for rice planting

The planting medium was a mixture of topsoil and manure, sun-dried for seven days in a 1:1 ratio. The mixture was placed in buckets and seedling trays, water added, and the mixture stirred several times until it became soft and muddy. Seeds of Aceh local rice and the Ciherang variety were selected, and the best seeds were chosen. The seeds were then soaked in water for 24 hours and germinated on gauze for 2 days. Seed sowing was then carried out in seedling trays filled with planting medium. The planting stage occurred 10 days after sowing (DAS). Seedlings of each strain were transplanted into buckets, with one seed per bucket.

The first fertilisation was at 10 days after planting (DAP) with NPK Phonska fertiliser at 0.93 g/pot (150 kg/ha) and urea at 0.31 g/pot (50 kg/ha). The second fertilisation was at 35 HST with NPK Phonska fertiliser at 0.62 g/pot (100 kg/ha) and urea at 0.62 g/pot (100 kg/ha). The final fertilisation was at 50 HST with NPK Phonska fertiliser at 0.62 g/pot (100 kg/ha). Maintenance of rice plants included replanting, weeding, and watering.

Harvesting begins when the grain is fully ripe. A characteristic of rice that is ready to be harvested is that the grains are hard and yellow.

Multiplication of isolates, preparation of suspensions and inoculation of bacteria

A bacterial isolate of *Xanthomonas oryzae* pv. *oryzae* (Xoo) was obtained from the Laboratory of Plant Protection, Faculty of Agriculture, Syiah Kuala University. Multiplication of the Xoo isolate is carried out in NA (nutrient agar) medium. 2 g of NA powder is added to an Erlenmeyer flask as a container for mixing, and 80 ml of aqueous solution is added. The Erlenmeyer flask is covered with aluminium foil, and the mixture is stirred until homogeneous, after which it is sterilised using an autoclave at 121 °C for 30 minutes. NA medium is poured into a sterile petri dish to a depth of 15 ml and left to set for a few minutes. The next step is to take an isolate from Xoo and streak it onto compacted NA medium. Subsequently, the new Xoo isolate is incubated in an incubator at 28 °C for 2 days.

The preparation of the Xoo suspension begins with a dilution procedure, and the density of Xoo is adjusted to 10⁸ cfu mL⁻¹. Xoo inoculation is performed at 35 days after planting (DAP). Inoculation is performed in the morning, between 8:00 and 9:00 am, using a Tudor sprayer that is applied to the entire leaf surface, particularly the underside and edges of rice leaves.

Observed parameters

Incubation period (days), incidence and severity of bacterial leaf blight (%)

The incubation period is calculated from the day after inoculation of Xoo bacteria until the first symptoms appear. Disease incidence is recorded 14 days after inoculation (DAI). Disease incidence is calculated using the following formula:

$$DI = \frac{a}{b} \times 100 \%$$

Note: DI = Disease incidence
 a = Total rice tillers showing symptoms of disease
 b = Total rice tillers observed

The severity of the disease is calculated once, i.e. one week before harvest (100 DAP), using the Townsend and Heuberger formula in Masnilah et al. (2020), as follows:

$$DSI = \frac{\sum (n \times v)}{N \times Z} \times 100\%$$

Note:
DSI = Disease severity index;
n = Number of leaves of each disease score;
v = Score of rating class;
N = Total number of leaves observed;
Z = Maximal disease index;

Tabel 1. Disease severity scale for bacterial leaf blight according to Chaudhry (1996) in Khan et al. (2015)

Disease Score		Lesion area (%)	Disease Category
0	0		Highly Resistant (HR)
1	>1-10 %		Resistant (R)
3	>11-30 %		Moderately Resistant (MR)
5	>31-50 %		Moderately Susceptible (MS)
7	>51-75 %		Susceptible (S)
9	>76-100 %		Highly Susceptible (HS)

The scoring of symptoms caused by rice leaves is shown in Figure 1.

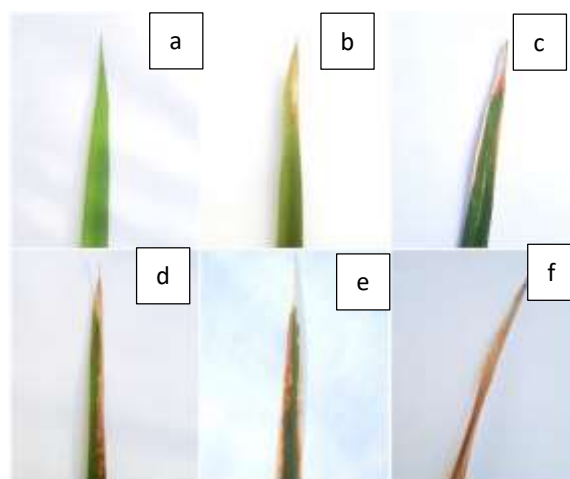


Figure 1. Symptoms on rice leaves were assessed using a severity scale for bacterial leaf blight, according to Chaubhry. (1996), scale 0 (0%) (a), scale 1 (>1–10 %) (b), scale 3 (>11–30 %) (c), scale 5 (>31–50 %) (d), scale 7 (>51–75 %) (e), and scale 9 (>76–100 %) (f)

Agronomic Character Parameters

Observation of agronomic characteristics includes the number of tillers per clump, the number of panicles per clump, the number of grains containing per panicle (grain) and the weight of grain per clump (gram).

Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA). If the F-test results indicate a significant effect, the analysis proceeds with the Duncan Multiple Range Test (DMRT) at the 5% significance level.

RESULT AND DISCUSSION

Incubation Period (Days), Incidence and Severity of Bacterial Leaf Blight (%)

The average incubation period of bacterial leaf blight in local rice lines is shown in Table 2. Table 2 shows that the incubation period of BLB disease is not significantly different between test lines. The average incubation period ranges from 4.33 to 5.66 days.

Table 2. Average incubation period, disease incidence and severity of bacterial leaf blight in local Acehnese rice lines due to *Xanthomonas oryzae* pv. *Oryzae* bacterial inoculation.

Treatments	Resistance test parameters		
	Incubation period (day)	Disease Incidence (%)	Disease Severity (%)
Tinggong (T.35.7.5.7.1)	4,83 ^a	56,46 ^a	37,72 ^{bc}
Tinggong (T.35.7.5.1.1)	4,66 ^a	48,05 ^a	26,43 ^a
Sambay Simeulue (US1)	5,50 ^a	45,34 ^a	29,02 ^a
Sambay Simeulue (US2)	4,83 ^a	40,89 ^a	26,25 ^a
Sigupai (UM69)	5,66 ^a	51,84 ^a	47,37 ^d

Sigupai (UM18)	5,33 ^a	48,10 ^a	39,02 ^c
Sikuneng Bireuen (UB5)	4,50 ^a	39,28 ^a	31,43 ^a
Sikuneng Bireuen (UB9)	4,33 ^a	45,04 ^a	31,25 ^a
Varietas Ciherang	4,50 ^a	57,03 ^a	32,17 ^{ab}

Note: Note: Numbers followed by the same letter in the same column are not significantly different in the DMRT test at the 5% level

The results of research Suryaningsih *et al.* (2023) showed that the Ciherang variety had an incubation period of 5 days after BLB disease inoculation. Carsono *et al.* (2021) also showed that the incubation period of BLB disease in rice lines expected between 3.94-4.92 days. The determination of the incubation period of BLB disease is based on the occurrence of symptoms due to inoculation with Xoo bacteria with a density of 10^8 cfu/ml. Symptoms that appear on the test rice plants can be seen in Fig 2.



Figure 2. Symptoms of bacterial leaf blight caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* on some local Aceh rice

Based on Figure 2a, the first symptoms of BLB disease are characterised by the appearance of grey lesions from the top to the edge of the rice leaves, either on one side or on both sides. Infected leaves will change colour from green to greyish brown and then dry out, as shown in Figure 2b. The results of research by Suryaningsih *et al.* (2023) indicate that the leaves of rice plants inoculated with Xoo bacteria will show symptoms such as the leaves turning yellowish and then greyish brown or drying out, starting from the edges of the rice leaves.

Disease development is influenced by the virulence of the pathogen, the host (rice plant), and the environment. In this study, the strain of Xoo bacteria used was not identified. It is known that the Ciherang variety is resistant to BLB disease with strain III, but is susceptible to strains IV and VIII (Susanto and Sudir. 2012). More virulent strains usually cause symptoms to appear faster, so the incubation period is shorter than with less virulent strains (Rahim *et al.* 2012). Environmental factors such as temperature and humidity affect the development of plant diseases. This experiment was conducted in a screen house with an average daily temperature of 26.8-32 °C and humidity of 57.5-89%,

which allowed the growth of Xoo on several local Acehnese rice strains and the Ciherang variety as a comparison. In agreement with Haque et al. (2022) and Sharma et al. (2017), temperatures of 25-34 °C with relative humidity of 70-84% can support the growth of BLB disease.

The average percentage of incidence of bacterial leaf blight in several local rice strains in Aceh is shown in Table 2. Based on the results of the statistical analysis, local Aceh rice lines do not differ significantly in the incidence of bacterial leaf blight. The average percentage of incidence of bacterial leaf blight 14 days after inoculation ranged from 39.28 to 57.03%. The high percentage is due to the large number of rice tillers that show symptoms of BLB disease. Hafiah et al. (2015) explained that the presence of plant resistance to pathogens, which is determined by genetic factors, can also affect the development of symptoms of BLB disease.

The average percentage of bacterial leaf blight severity in several local Aceh rice strains is shown in Table 2. Analysis of variance of local Aceh rice lines showed a very significant difference in BLB disease severity. The lowest percentage of bacterial leaf blight severity was found in Aceh Sambay Simeulu (US2) at 26.25%, which was not significantly different from Tinggong rice (T.35.7.5.1.1) at 26.43%, Sambay Simeulue (US1) at 29.02%, Sikuneng Bireuen (UB9) at 31.25%, Sikuneng Bireuen (UB5) at 31.43%, and Ciherang variety at 32.17%. However, it was significantly different from Tinggong rice (T.35.7.5.7.7.1) at 37.72%, Sigupai (UM18) at 39.02%, and Sigupai (UM69) at 47.37%, which had the highest percentage of severity.

According to Chaudhry (1996), the resistance categories are presented in Table 1. The rice varieties included in the moderately resistant category are Sambay Simeulue (US2), Tinggong (T.35.7.5.1.1) and Sambay Simeulue (US1). The rice varieties in the moderately susceptible category are Sikuneng Bireuen (UB9), Sikuneng Bireuen (UB5), Ciherang variety (comparison), Tinggong (T.35.7.5.7.1), Sigupai (UM18) and Sigupai (UM69).

The resistance category of Sambay Simeulue Mutant Rice to BLB disease is moderate. It is suspected to carry a resistance gene due to gamma-ray radiation. Sigupai (UM18) and (UM69) rice are also included in irradiated breeding rice, but show a resistance category that is moderately susceptible to BLB disease. These differences in resistance are influenced by different genes. The results of the study by Marlina et al. (2020), which tested local Aceh smart rice lines derived from radiation mutations against BLB disease, indicate that there are no rice lines that are resistant to BLB disease.

Tinggong rice (T.35.7.5.1.1), Tinggong (T.35.7.5.7.1), Sikuneng Bireuen (UB9) and Sikuneng Bireuen (UB5) are the result of crossing local Aceh rice with IRBB27 isogenic rice derived from IRRI and are known to contain the resistance gene (Xa-27) against bacterial leaf blight (Zakaria et al. 2021). Based on the results of this study, Tinggong rice (T.35.7.5.1.1) is included in the moderately resistant category, while Tinggong (T.35.7.5.7.1) is in the moderately susceptible category. Previous research by

Khatimah et al. (2021) found that the genotypes T.35.7.5.1 and T.35.7.5.7 (derived BC3F2) showed a resistant reaction to Xoo strains IV and VIII, based on the length of the lesions on rice leaves showing symptoms. The difference in categories is suspected because the bacterial strains inoculated in several test strains can break the Xa-27 gene contained in the rice. Tasliah (2012) states that Xoo bacteria have many strains and that those strains can change, as a result of which previously resistant rice varieties can become susceptible.

Parameters of the agronomic character of Aceh's local rice

The average values of the local rice agronomic variables in Aceh are presented in Table 3.

Table 3. Average agronomic character parameters: number of tillers per clump, number of panicles per clump, number of filled grains per panicle, and grain weight per clump

Treatments	Agronomic character parameters			
	Number of tillers per clump (Stems)	Number of panicles per clump (Panicles)	Number of filled Grains per panicle (Grain)	Grain weight per clump (Gram)
Tinggong (T.35.7.5.7.1)	21,50 ^c	27,5 ^c	135,72 ^{abc}	59,15 ^b
Tinggong (T.35.7.5.1.1)	17,67 ^c	22,67 ^{bc}	189,78 ^{cd}	66,86 ^b
Sambay Simeulue (US1)	17,83 ^c	23,5 ^{bc}	206,49 ^d	75,92 ^b
Sambay Simeulue (US2)	14,17 ^{bc}	15,33 ^{ab}	120,37 ^{ab}	44,46 ^{ab}
Sigupai (UM69)	7,50 ^{ab}	9,33 ^a	118,12 ^{ab}	35,93 ^{ab}
Sigupai (UM18)	5,00 ^a	6,5 ^a	100,41 ^a	21,16 ^a
Sikuneng Bireuen (UB5)	16,50 ^c	20,17 ^{bc}	191,13 ^{cd}	74,44 ^b
Sikuneng Bireuen (UB9)	18,67 ^c	20,83 ^{bc}	171,02 ^{bcd}	62,59 ^b
Varietas Ciherang	20,50 ^c	24,17 ^{bc}	146,00 ^{abcd}	60,73 ^b
Note:	Numbers followed by the same letter in the same column are not significantly different in the DMRT test at the 5% level			

According to the analysis of the variety of treatments for several Aceh local rice strains, there was a very significant difference in the number of rice plant tillers. Based on Table 3, the average number of tillers was highest in Tinggong rice (T.35.7.5.7.1) at 21.5 stems, which was not significantly different from the Ciherang variety (20.50 stems), Sikuneng Bireuen (UB9) (18.67 stems), Sambay Simeulue (US1) (17.83 stems), Tinggong (T.35.7.5.1.1) (17.67 stems), and Sambay Simeulue (US2) (14.17 stems), but it was significantly different from Sigupai (UM69) (7.50 stems) and Sigupai (UM18) (5.00 stems).

The number of rice tillers varies between lines or varieties. Some varieties produce many tillers quickly, while others produce few tillers very slowly. According to Karlinah et al. (2023), the formation of rice tillers is influenced by genetic and environmental factors, such as temperature, water, sunlight intensity and nutrients. Setyowati et al. (2018) reported that local rice in Aceh Tinggong has a number of tillers reaching 38.75 stems per clump. Aceh Sigupai mutant M5 rice, given organic fertilizer treatment, had 10.48-11.32 tillers (Safrizal et al. 2024).

Based on the results of the statistical analysis, the local rice strain in Aceh shows a very significant difference in the number of panicles per clump. Table 3 shows that the Aceh Tinggong local rice (T.35.7.5.7.1) has the highest number of panicles per clump at 27.50, which is not significantly different from the Ciherang (Comparator) (24.17 panicles), Sambay Simeulue (US1) (23.5 panicles), Tinggong (T.35.7.5.1.1) (22.67 panicles), Sikuneng Bireuen (UB9) (20.83 panicles), and Sikuneng Bireuen (UB5) (20.17 panicles), but is significantly different from Sambay Simeulue (US2) (15.33 panicles), Sigupai (UM69) (9.33 panicles), and Sigupai (UM18), which have the fewest panicles (6.5 panicles).

Each rice line has a different genetic potential to produce the number of panicles per clump. The study by Efendi et al. (2020) reported that the F3 strain from the cross between Sikuneng and IRBB27 can produce 10.3-39.2 panicles per clump. The number of panicles per clump in rice plants is related to the number of rice saplings that grow. Asis et al. (2021) state that the number of rice tillers greatly affects plant productivity, because the number of panicles that produce grain is proportional to the number of seedlings that grow.

Based on the results of variance analysis, local Aceh rice strains significantly affect the number of filled grains per panicle. According to Table 3, the local Aceh rice strain with the highest average number of filled grains per panicle is Sambay Simeulue (US1) at 206.49 grains. This is not significantly different from Sikuneng Bireuen (UB5) (191.13 grains), Tinggong (T.35.7.5.1.1) (189.78 grains), Sikuneng Bireuen (UB9) (171.02 grains) and Ciherang varieties (146 grains), but is significantly different from Tinggong (T.35.7.5.7.1) (135.72 grains), Sambay Simeulue (US2) (120.37 grains), Sigupai (UM69) (118.12 grains) and Sigupai (UM18), which has the lowest number of filled grains at 100.41 grains.

The number of filled grains per panicle is related to the number of panicles per clump. Some lines produce more panicles with fewer filled grains, while others have fewer panicles but with more grains per panicle. This is related to the presence of late-appearing and less productive panicles. Sitinjak and Idwar (2015) added that genetic factors such as panicle length and panicle branches can also affect the number of filled grains per panicle. According to Suprayogi et al. (2021), grain weight per clump is heavier when more filled grains are present in one panicle.

Based on the results of the variance analysis, Aceh local rice lines significantly affect the weight of grain per clump. Table 3 shows that the highest grain weight per clump was observed in the Sambay Simeulue strain (US1), at 75.92 g, which is not significantly different from any of the treatments but is significantly higher than that of the Sigupai rice strain (UM18), which has the lowest grain weight per clump at 21.16 g.

There is an effect between the number of filled grains per clump and the weight of grain per clump. The more the number of filled grains per panicle, the heavier the grain weight per clump. The low weight of grain per clump in Sigupai rice strains is influenced by the incidence and severity of BLB

disease caused by Xoo bacteria inoculation. Yuliani and Sudir (2017) stated that BLB disease attacks in the generative phase can affect the grain-filling process, resulting in hollow grains. Djatmiko and Fatichin (2009) added that low disease intensity is usually followed by high grain-filling weight.

CONCLUSION

The results of this study indicate that the rice lines Sambay Simeulue (US2), Tinggong (T.35.7.5.1.1) and Sambay Simeulue (US1) are classified as moderately resistant, while the Sikuneng Bireuen (UB9), Sikuneng Bireuen (UB5), Tinggong (T.35.7.5.7.1), Sigupai (UM18) and Sigupai (UM69) rice strains are classified as moderately susceptible to bacterial leaf blight. Further research is needed on the level of resistance of local Acehnese rice strains, derived from cross-breeding and gamma radiation, to bacterial leaf blight across several strains of Xoo bacteria.

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