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Resistance of Doubled Haploid Rice Lines with Green Super Rice Characters to Bacterial Leaf Blight

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Abstract

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a significant disease attacking rice crops worldwide. This disease attacks at various stages of plant growth and causes significant yield loss. Breeding new varieties resistant to BLB is important to support sustainable agriculture in the future. This study aimed to identify new superior green super rice (GSR) lines resistant to BLB disease. The experiment evaluated the resistance of lowland rice lines obtained from anther culture using a factorial randomized complete block design. The 1st factor was genotype, consisting of 20 lines, 2 checks of commercial varieties (Inpari 42 Agritan GSR and Inpari 18), a resistant check (Conde), and a susceptible check (Taichung Native 1). The 2nd factor was BLB pathotypes (i.e., III, IV, and VIII). Quantitative data on disease severity and severity index were analyzed using analysis of variance and t-Dunnett's test at 5% level. The results showed that the interaction between genotype and pathotype affected the disease severity and severity index in both growth phases. The tested lines exhibited varying resistance, from susceptible to resistant, to BLB. Four lines (SN 11, 13, 57, and 58) showed moderate to resistant criteria for BLB disease of 3 pathotypes in both growth phases. The selected lines can be used as a source of parents for breeders and candidates for new superior varieties with BLB resistance properties to support the reduction of synthetic chemical bactericide inputs and control BLB disease. However, further field evaluations are necessary to assess their performance.

Keywords: anther culture; disease severity; rice breeding; *Xanthomonas oryzae* pv. *oryzae*

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INTRODUCTION

Rice is a staple food consumed by more than 50% of the world's population (Jiang et al., 2020) and is a staple food for the majority of people in Indonesia (Rachman et al., 2022). In 2021, domestic rice production decreased by around 0.43% from the previous year, and the downward trend continued until 2023 (Statistics Indonesia, 2024a). Domestic production has been unable to meet demand and has sufficient reserves, so the government has taken steps to import rice. Based on data from Statistics Indonesia (2024b), over the last 3 years, Indonesia's rice imports amounted to 356,286 tons in 2019, then increased to 407,741 tons in 2021 and 429,207 tons in 2022.

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Gram-negative bacteria, specifically *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) are responsible for causing bacterial leaf blight (BLB) disease, which affects rice plants (Ashwini et al., 2021; Khannetah et al., 2021). This disease attacks most rice-growing countries worldwide (Azizi and Lau, 2022). This BLB can cause rice yield losses of 10 to 20% (Duy et al., 2021) and even up to 50% (Liu et al., 2014) and is economically damaging worldwide (Sanya et al., 2022). Subehi et al. (2020) stated that BLB attacked rice plantations in Indonesia around 46,185 ha in 2018, 39,540 ha in 2019, and 38,485 ha in 2020.

BLB pathotypes are grouped based on different levels of virulence. Noer et al. (2018) confirmed that in Indonesia, 6 pathotypes were found, while Asysyuura et al. (2017) reported that pathotype XII was found in Pangkep and Takalar Regencies of South Sulawesi, Indonesia. However, the dominant ones that develop and attack rice production centers in Indonesia are 3 pathotypes: III, IV, and VIII (Sudir and Yuliani, 2016). Pathotype III predominantly attacks rice centers in South Sulawesi, South Sumatra, and Yogyakarta. Pathotype IV predominantly attacks the provinces of North Sumatra, Lampung, and West Nusa Tenggara (Sudir and Yuliani, 2016). Pathotype VIII attacks the provinces of Banten, West Java, Central Java, and East Java (Sudir and Yuliani, 2016). In China, pathotype IX is a new pathotype growing rapidly (Chen et al., 2018). Deng et al. (2016) reported that 45 pathotypes of 80 highly virulent *Xoo* pathotypes were identified based on identification results from 1986 to 2011 in Taiwan. Of the 300 *Xoo* isolates collected from 17 districts in Pakistan, they were grouped into 29 pathotypes, with pathotype 1 being the most virulent of all the other pathotypes (Arshad et al., 2017). In India, 400 isolates collected were grouped into 22 pathotypes. Pathotypes 1 and 2 were less virulent, while pathotypes 3 to 22 were highly virulent (Yugander et al., 2017).

Suppression of bacterial growth can be done in various ways, such as the use of antagonistic bacteria (Yasmin et al., 2016), fluridone induction to suppress abscisic acid (Ding et al., 2019), bioengineering approaches (Ahmed et al., 2022), and breeding resistant variety with conventional and molecular approaches (Chukwu et al., 2019). Breeding of superior varieties that are responsive to nitrogen can increase rice production. However, continuous use of nitrogen combined with wet environmental conditions can increase disease incidence. Synthetic disease control can increase adverse environmental impacts (Pradhan et al., 2015). Planting resistant varieties may reduce yield losses due to BLB disease, thus supporting environmentally friendly agriculture (Ji et al., 2018).

One of the rice breeding that supports sustainable agriculture is the development of green super rice (GSR). GSR has several characteristics, such as increased yield per unit area, good quality, resistance to major pests and diseases, efficient nutrient absorption (Jewel et al., 2019), tolerance to drought (Yu et al., 2020), salinity (Amanat et al., 2022), high-temperature stresses (Zafar et al., 2022), and increased adaptability to future climate change (Li and Ali, 2017; Zafar et al., 2022). GSR breeding can be done using biparental crosses (Nurhidayah et al., 2023), backcrossing to produce rice that is resistant to pests and diseases (Ali et al., 2021), pyramiding genes using Marker-assisted Selection (MAS) (Chukwu et al., 2019), identifying of Quantitative Trait Loci (Huerta et al., 2021), genomic breeding approaches (Yu et al., 2020), and CRISPR-Cas9 (Zafar et al., 2020). Chemical residues in pesticides will cause environmental pollution in soil, water, and air. Apart from that, it will be toxic to other organisms, such as beneficial insects and non-target plants (Tudi et al., 2021). GSR varieties resistant to BLB disease will benefit farmers in the future because using bactericides becomes more efficient in supporting environmentally friendly agriculture.

The Indonesian Ministry of Agriculture released the Inpari 42 Agritan GSR, Inpari 43 Agritan GSR, and Inpari 46 GSR TDH varieties from 2016 to 2019 (Susanto et al., 2022). However, the breeding of GSR varieties that are more resistant to the 3rd pathotype needs further study to obtain resistant varieties. Therefore, the researchers used the GSR varieties as biparental cross parents in this study (Inpari 42 GSR × B22-1, B-22-1 × Inpari 42 Agritan GSR, Inpari 42 Agritan GSR × Inpari 46 GSR TDH, Inpari 42 Agritan GSR × Inpari IR Nutri Zinc, and Inpari 42 Agritan GSR × Bionil 6–1). Based on these problems, this study aims to evaluate resistance to BLB disease in doubled haploid (DH) GSR lines obtained from anther culture. This study aimed to identify new superior GSR lines resistant to BLB disease. These genotypes may contribute to reducing the use of synthetic chemical bactericides in the future and suppressing the spread of BLB disease.

MATERIALS AND METHOD

Plant materials and field planting

The experiment was carried out in the greenhouse of the Center for Instrument Standard Testing of Biotechnology and Agricultural Genetic Resources, Bogor, West Java Province, at an altitude of 220 meters above sea level (m asl) with coordinates of 106°47'06.7" E and 6°34'32.2" S. The research was carried out from September to December 2023.

The research materials used were rice genotypes from the previous selection of 20 lines (Nurhidayah et al., 2023), 2 check commercial varieties (Inpari 42 Agritan GSR and Inpari 18), the Taichung Native 1 (TN-1) variety as a susceptible check, and the Conde variety as a resistant check (Table 1). Other materials include soil, manure, and NPK fertilizer.

The seeds were placed in a petri dish, soaked in sterile water for 24 hours, and then incubated for 5 days. Next, the germinating seeds were transplanted into pots filled with mud and manure in a ratio of 10:1. The seedlings were planted at a distance of 3 cm x 3 cm and maintained until the plants were ready to be inoculated. Plants were fertilized using NPK fertilizer at a dosage of 3 g plant^{-1} which was applied at 1, 4, and 8 weeks after planting (WAP). Plants were watered in each plot until the soil was submerged. Weeds that grew were removed manually. Plants were ready for inoculation at 30 and 60 days after planting.

Experimental design

The research employed a factorial randomized complete block design with 2 main factors: genotype and BLB pathotype. The genotype factor comprised 24 genotypes while the BLB pathotype factor consisted of 3 pathotypes (III, IV, and VIII), resulting in 72 treatment combinations. Each treatment combination was replicated 3 times, leading to 216 experimental units. Five plants were grown within each unit, and 2 leaves per plant were sampled, totaling 10 leaves per experimental unit.

Preparation of *Xoo* suspension and bacterial inoculation

The preparation of *Xoo* suspension followed the method of Yuriyah et al. (2021). Bacterial inocula of pathotypes III, IV, and VIII were cultured using Wakimoto plus ferrous sulfate (WF-P) agar media in a petri dish for 48 hours at 28 °C until a single colony grew. These colonies were then taken using a sterile tube into an erlenmeyer flask containing 10 ml of sterile water and were agitated to produce a suspension of *Xoo* conidia. The resulting suspension was stirred thoroughly and the concentration was adjusted to 10^9 cells ml^{-1}.

The *Xoo* suspension was inoculated using the scissors (clipping) method. Sterile scissors were dipped in the bacterial suspension containing the *Xoo* bacterial pathotype and then inoculated on the leaf by cutting the tip of around 1.5 cm. The experiment used 30 fully opened rice leaves aged 30 days in the vegetative phase and 60 days in the generative phase. After inoculation, the greenhouse humidity was maintained at around > 65% by misting with a sprinkler until 14 days post-inoculation. Observations were conducted on the 14th day after inoculation by measuring the length of leaves infected with BLB, with the leaf length observed in each treatment.

Variable evaluation

Disease severity was measured by measuring the length of the affected leaf (cm) divided by the observed leaf length (cm) × 100 (IRRI, 2014; Biswas et al., 2021). Data on the percentage of disease severity for each leaf were then used to calculate the percentage of disease severity index

Table 1. Lowland rice lines resulting from anther culture tested

No.	Code	Genotype	No.	Code	Genotype
1.	SN2	FS1-69-1-1	13.	SN28	FS6-13-1-1
2.	SN3	FS2-6-1-1	14.	SN32	FS6-29-1-1
3.	SN5	FS2-6-1-3	15.	SN40	FS6-32-2-1
4.	SN9	FS5-5-1-2	16.	SN51	FS6-56-1-1
5.	SN11	FS5-11-1-2	17.	SN57	FS8-6-1-1
6.	SN12	FS5-11-1-3	18.	SN58	FS8-6-1-2
7.	SN13	FS5-11-2-1	19.	SN59	FS8-6-1-3
8.	SN14	FS5-20-1-1	20.	SN60	FS8-6-1-4
9.	SN15	FS5-25-1-1	21.	Commercial variety	Inpari 42 Agritan GSR
10.	SN18	FS5-33-1-3	22.	Commercial variety	Inpari 18
11.	SN20	FS5-33-1-5	23.	Susceptible check	Taichung Native-1 (TN-1)
12.	SN25	FS6-4-1-2	24.	Resistant check	Conde

$$\text{DSI (\%)} = \text{Sum}\frac{\text{(Number of affected leaves on each scale} \times \text{score severity of leaf)}}{\text{(Total leaves observed} \times \text{highest severity score)}} \text{ x } 100 \quad (1)$$

(DSI) (IRRI, 2014; Bock et al., 2020). The DSI (%) was calculated using Equation 1.

The level of disease resistance was measured based on calculating the disease severity for each genotype and then grouping resistance based on the Standard Evaluation System of Rice (SES) (IRRI, 2014) which is presented in Table 2.

Table 2. Criteria of BLB disease resistance based on disease severity

Scale	Disease severity (%)	Level of resistance
0	0	HR = Highly resistance
1	1-6	R = Resistance
3	> 6-12	MR = Moderately resistance
5	> 12-25	MS = Moderately susceptible
7	> 25-50	S = Susceptible
9	> 50-100	HS = Highly susceptible

Source: IRRI (2014)

Data analysis

The disease severity and DSI data sets were subjected to analysis of variance and further tested by t-Dunnett's test at level 0.05 using SAS on Demand for Academics software (https://welcome.oda.sas.com/). BLB resistance levels were tabulated using Microsoft Excel and analyzed descriptively.

RESULTS AND DISCUSSION

Performance of DH GSR lines against BLB disease in the vegetative phase

As presented in Table 3, the analysis of variance revealed that genotype significantly affected disease severity and DSI. The pathotype significantly impacted disease severity and DSI. The genotype × pathotype interaction significantly influenced disease severity and DSI. Coefficient of variation (CV) disease severity had 9.97% and DSI had 5.74%. A further t-Dunnett's test at the 5% significance level was conducted for the factors that showed significant effects (Table 4).

The disease severity of lines against BLB pathotype III was 1.88 to 23.26%, pathotype IV was 1.79 to 23.80% and pathotype VIII was 7.76 to 18.75%. Four lines (SN 3, 9, 25, and 28) showed no significant difference from the susceptible check variety TN-1 variety when exposed to BLB pathotype III during the vegetative phase. Sixteen other lines and Conde were significantly better than the TN-1 variety to BLB pathotype III. For BLB pathotype IV, line SN 15 did not differ from TN-1, while SN 3 did not differ from TN-1 in response to BLB pathotype VIII. The Inpari 18 variety was not significantly different from the susceptible check variety TN-1 in pathotypes III and IV. Meanwhile, several lines significantly exhibited lower disease severity than the susceptible check variety TN-1, BLB pathotypes IV and VIII (Table 4). Evaluation of the source of disease resistance in genotypes depends on accurate measurement of disease severity estimates (Habib et al., 2022).

The DSI varied across the tested lines. For pathotype III, the DSI ranged from 9.70 to 61.66%; pathotype IV ranged from 10.15 to 48.98%; and pathotype VIII ranged from 27.54 to 51.29%. DSI of 16 lines, Inpari 42 Agritan GSR, and Conde were significantly different from the TN-1 as a susceptible variety, except for 4 lines (SN 3, 9, 25, and 28) and Inpari 18 which were insignificant from TN-1 to against BLB pathotype III. The DSI of all lines, Inpari 42 Agritan GSR, and Conde was significantly lower than the susceptible check variety TN-1 except SN 15 and Inpari 18, which was insignificant against TN-1 variety on BLB pathotype IV. The DSI of the 11 lines, Inpari 18, and Conde significantly differed from the TN-1 variety against BLB pathotype VIII.

Based on the screening results of the 20 rice lines tested, resistance scores ranging from 1 to 5 were obtained for all pathotypes, indicating varying resistance levels to BLB, from moderately susceptible to resistant (Table 5). Six lines (SN 3, 9, 18, 25, 28, and 51) were classified

Table 3. Analysis of variance on disease severity and severity index in the vegetative phase

Traits	Block	Genotype	Pathotype	Genotype × Pathotype	CV (%)
Disease severity	0.149*	0.482**	0.508**	0.149**	9.97
DSI	0.347**	0.210**	0.574**	0.070**	5.74

Note: *, ** = significant at 0.05 and 0.01 levels, respectively; data were transformed with log transformation

Table 4. Disease severity and severity index for BLB disease on DH GSR lines from anther culture in the vegetative phase

Genotype	Disease severity (%)			DSI (%)		
	P III	P IV	P VIII	P III	P IV	P VIII
SN 2	9.48*	11.57*	10.96*	26.10*	34.41*	38.90*
SN 3	19.35ns	14.57*	18.75ns	42.99ns	31.87*	51.29ns
SN 5	2.11*	9.26*	16.34*	13.39*	29.06*	47.13ns
SN 9	22.22ns	16.98*	11.39*	48.60ns	35.75*	34.67*
SN 11	2.63*	1.79*	8.13*	13.80*	10.15*	31.87*
SN 12	2.49*	4.86*	7.76*	13.80*	16.34*	27.54*
SN 13	4.90*	4.82*	10.23*	25.51*	16.72*	37.73*
SN 14	3.63*	19.95*	14.45*	16.09*	41.05*	41.69ns
SN 15	8.98*	23.80 ns	9.62*	38.02*	48.98ns	34.67*
SN 18	13.80*	5.33*	11.84*	33.37*	16.47*	38.90*
SN 20	11.31*	3.49*	12.98*	37.44*	14.45*	43.65ns
SN 25	23.26ns	13.18*	16.98*	61.66ns	35.21*	46.77ns
SN 28	15.85ns	13.08*	15.25*	44.67ns	37.73*	43.32ns
SN 32	7.70*	14.13*	14.45*	36.31*	36.59*	45.36ns
SN 40	4.61*	11.93*	12.59*	14.68*	30.20*	41.69ns
SN 51	12.49*	12.21*	11.93*	36.31*	30.43*	43.32ns
SN 57	1.88*	10.63*	10.31*	9.70*	27.12*	37.44*
SN 58	6.26*	4.61*	9.77*	16.47*	17.25*	33.11*
SN 59	9.33*	9.19*	8.45*	18.06*	27.75*	29.29*
SN 60	10.47*	4.30*	12.88*	29.97*	15.25*	40.43*
Inpari 42 Agritan GSR	6.61*	8.32*	13.80*	19.20*	23.62*	47.13ns
Inpari 18	17.25ns	29.29ns	9.33*	40.43ns	57.54ns	34.41*
TN 1 (Susceptible check)	26.10	38.02	33.37	61.19	74.13	66.07
Conde (Resistant check)	3.80*	5.01*	2.29*	22.22*	14.45*	11.84*
Mean	10.27	12.10	12.66	30.00	30.11	39.51

Note: P = Pathotype, * = Significantly different, ns = Not significantly different to susceptible check (TN-1) based on t-Dunnett's test at 0.05 level

as moderately susceptible to BLB pathotype III, similar to the Inpari 18 variety. Six other lines (SN 2, 15, 20, 32, 59, and 60) were categorized as moderately resistant, aligning with their parent variety Inpari 42 Agritan GSR, which exhibits moderate resistance to BLB pathotype III. Eight lines (SN 5, 11, 12, 13, 14, 40, 57, and 58) were classified as resistant to BLB pathotype III during the vegetative phase, comparable to the Conde variety, which served as a resistance check.

Pathotype IV BLB inoculated on the tested lines was on a scale of 1 to 5 from moderately susceptible to resistant (Table 5). However, the lines tested for pathotype IV had more criteria for being moderately susceptible (8 lines = 40%) than pathotype III (30%). The criteria for moderately resistant were 5 lines (SN 2, 5, 40, 57, and 59) or 25% of the line tested. Criteria for resistance to BLB pathotype IV in a vegetative phase were 7 lines (SN 11, 12, 13, 18, 20, 58, and 60) or 35% of the line tested.

Resistance to BLB pathotype VIII during the vegetative phase was observed on only 2 scales (3 and 5) corresponding to 2 resistance criteria: moderately susceptible and moderately resistant (Table 5). Nine lines (45%) were classified as moderately susceptible, which is the same percentage as for pathotype IV (45%) but higher than for pathotype III (30%). Eleven lines (SN 2, 9, 11, 12, 13, 15, 18, 51, 57, 58, and 59) or 55% of the line tested were identified as moderately resistant, similar to the Inpari 18 variety. This population has no resistant and highly resistant lines against BLB pathotype VIII. This finding aligns with the research by Putri et al. (2023), which reported that the 12 genotypes tested were generally more susceptible to pathotype VIII compared to the other 2 pathotypes.

Among the 20 lines tested in this study, 6 lines (SN 11, 12, 13, 57, 58, and 59) were identified as moderately resistant to resistant against all 3 BLB pathotypes (III, IV, and VIII) (Table 5). Khannetah et al. (2021) stated that such genetic resources have the potential to serve as donors for developing rice varieties resistant to BLB. The TN-1 variety consistently showed susceptibility

Table 5. Score and criteria for BLB disease resistance in DH GSR lines in the vegetative phase

Genotype	Pathotype III		Pathotype IV		Pathotype VIII	
	Score	Criteria	Score	Criteria	Score	Criteria
SN 2	3	MR	3	MR	3	MR
SN 3	5	MS	5	MS	5	MS
SN 5	1	R	3	MR	5	MS
SN 9	5	MS	5	MS	3	MR
SN 11	1	R	1	R	3	MR
SN 12	1	R	1	R	3	MR
SN 13	1	R	1	R	3	MR
SN 14	1	R	5	MS	5	MS
SN 15	3	MR	5	MS	3	MR
SN 18	5	MS	1	R	3	MR
SN 20	3	MR	1	R	5	MS
SN 25	5	MS	5	MS	5	MS
SN 28	5	MS	5	MS	5	MS
SN 32	3	MR	5	MS	5	MS
SN 40	1	R	3	MR	5	MS
SN 51	5	MS	5	MS	3	MR
SN 57	1	R	3	MR	3	MR
SN 58	1	R	1	R	3	MR
SN 59	3	MR	3	MR	3	MR
SN 60	3	MR	1	R	5	MS
Inpari 42 Agritan GSR	3	MR	3	MR	5	MS
Inpari 18	5	MS	7	S	3	MR
TN 1 (Susceptible check)	7	S	7	S	7	S
Conde (Resistant check)	1	R	1	R	1	R

Note: R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible

to all pathotypes, while the Conde variety demonstrated consistent resistance. This finding is consistent with the research by Yuriyah et al. (2021) that Conde exhibited symptoms of resistance to the 3 pathotypes during the vegetative phase. Conde's resistance is attributed to the presence of 2 genes, Xa4 and Xa7, which confer resistance to all 3 BLB pathotypes.

Meanwhile, the Inpari 42 Agritan GSR variety exhibited the criteria of moderately resistant response to BLB pathotypes III and IV and moderately susceptible to pathotype VIII. This finding is consistent with the rice descriptor released by the Ministry of Agriculture of Indonesia (2021) that the Inpari 42 variety has resistance with the criteria of moderately resistant to BLB pathotypes III and moderately susceptible to pathotypes VIII (Sastro et al., 2021).

According to Agrios (2005), pathogens infecting leaves can lead to increased transpiration. This occurs because the cuticle that protects the leaves becomes damaged, the stomata do not function, and leaf permeability increases. The *Xoo* pathogen is involved in the degradation of the plant cell wall, secretion processes, lipopolysaccharide production, and the detoxification defense mechanisms within the host plant (Quibod et al., 2020). Bacteria can grow in the intercellular space of the mesophyll 5 days after infiltration inoculation, which can cause yellow lesions and exudate formation at the leaf inoculation site (Cao et al., 2020).

The disease incidence and severity are determined by 3 factors: plant (host), pathogen (virulence and amount of inoculum), and environment (Agrios, 2005). Infection and disease severity are influenced by pathogen and host interactions, soil conditions, soil microbiomes (Singh et al., 2023), and suitable climate variables (Saputra et al., 2023) thus reducing rice productivity. Wijayanto et al. (2024) reported that high bacterial leaf spot disease attacks can be caused by high rainfall, warm temperatures, high humidity, rainwater splashes, wind, and irrigation water. Hamid and Ghazanfar (2022) emphasized the need for integrated disease management and the latest utilization of combined control strategies, including enhancing host defense mechanisms, intercropping, resistant varieties, and crop rotation.

Performance of DH GSR lines against BLB disease in the generative phase

The variance analysis shows that genotype, pathotype, and the interaction of genotype × pathotype significantly affected the disease severity and DSI of BLB disease in the generative phase (Table 6). Coefficient of variation (CV) disease severity had 10.68% and DSI had 5.75%. Further analysis was then carried out on the existence of this influence using the t-Dunnett's test at 0.05 level.

The disease severity of GSR rice lines to pathotype III ranged from 0.50 to 11.66%. The parent Inpari 42 Agritan GSR had a severity of 1.42%, the commercial variety Inpari 18 had 8.72%, the susceptible check variety TN-1 had 35.97%, and the resistant check variety Conde had 3.14% (Table 7). The tested lines showed significant differences from the susceptible check variety TN-1. The disease severity of pathotype IV in the generative phase in the lines tested ranged from 2.81 to 37.67%, Inpari 42 Agritan GSR 6.39%, Inpari 18 19.0%, susceptible check TN-1 38.93%, and resistant check Conde variety 2.23%. Five lines (SN 2, 9, 12, 15, and 20) were not significantly different from the susceptible check variety TN1 pathotype IV. Meanwhile, 15 other lines had significantly better or lower disease severity than the susceptible check variety TN-1. The disease severity of pathotype VIII in the lines ranged from 4.65 to 9.71%, Inpari 42 Agritan GSR 10.65%, Inpari 18 12.37%, the susceptible check variety TN-1 18.34%, and the resistant check variety Conde 0.86%. The Inpari 18 and Inpari 42 Agritan GSR varieties were not significantly different from the TN-1 susceptible check. All tested lines exhibited better resistance or lower disease severity than the susceptible variety TN-1.

The DSI of pathotype III in the lines tested ranged from 3.31 to 27.35%. Meanwhile, Inpari 42 GSR had a DSI of 9.04%, Inpari 18 had 15.91%, the susceptible check variety TN-1 had 66.12%, and the resistant check variety Conde had 12.73% (Table 7). All tested lines demonstrated a significantly lower DSI compared to the susceptible check variety TN-1, indicating better resistance.

The DSI of pathotype IV in the tested lines ranged from 13.92 to 69.93%. Inpari 42 Agritan GSR had a DSI of 20.32%, Inpari 18 had 40.86%, TN-1 had 48.57%, and the Conde variety had 10.26%. Ten tested lines (SN 3, 5, 11, 13, 18, 28, 32, 51, 57, and 58) were significantly better or had a lower DSI percentage than the TN-1 variety. Meanwhile, the other 10 lines (SN 2, 9, 12, 14, 15, 20, 25, 40, 59, and 60) had the same percentage of DSI as the TN-1 susceptible check (Table 7).

The DSI of pathotype VIII in the tested lines ranged from 14.87 to 37.55%. Meanwhile, the DSI of Inpari 42 Agritan GSR was 37.01%, Inpari 18 was 39.32%, TN-1 was 57.32%, and Conde was 5.89% (Table 7). All tested lines, except SN 3, had a lower DSI than the TN-1 susceptible check. Disease severity and DSI in the generative phase were generally lower than in the vegetative phase, except for pathotype IV, which showed a higher DSI in the generative phase compared to the vegetative phase (Table 4 and 7).

As presented in Table 8, the lines tested exhibit a range of resistance levels to each BLB pathotype, from susceptible to resistant. This finding is consistent with the report of Khannetah et al. (2021), that the lines tested showed different resistance among the 100 lines tested: 4 were resistant, 34 were moderately resistant, 49 were moderately susceptible, and 13 were susceptible. In this study, resistance to BLB pathotype III was on a scale of 1 to 3, with the criteria ranging from moderately resistant to resistant for all lines tested, Inpari 42 Agritan GSR, and the resistant check variety Conde. Three lines (15%) received a score of 3, indicating moderately resistant, while 17 lines (85%) achieved a score that categorized them as resistant (Table 8).

Resistance to BLB disease pathotype IV was assessed on a scale of 1 to 7, encompassing criteria from susceptible to resistant. Three lines (SN 12, 15, and 20) were susceptible, or around 15% of the total genotype population tested. Six lines were moderately susceptible (30%). Nine lines (SN 3, 5, 11, 13, 18, 51, 57, 58, and 60) were moderately resistant (45%) same as Inpari

Table 6. Analysis of variance on disease severity and severity index in the generative phase

Traits	Block	Genotype	Pathotype	Genotype × Pathotype	CV (%)
Disease severity	0.129^{*}	0.619^{**}	8.761^{**}	0.286^{**}	10.68
DSI	0.025^{ns}	0.269^{**}	0.458^{**}	0.136^{**}	5.75

Note: *, ** = Significant at 0.05 and 0.01 levels, respectively, ^{ns} = Not significant; data were transformed with log transformation

Table 7. Disease severity and severity index for BLB disease on DH GSR lines from anther culture in the generative phase

Genotype	Disease severity (%)			DSI (%)		
	P III	P IV	P VIII	P III	P IV	P VIII
SN 2	1.41*	21.59ns	8.78*	10.84*	36.36ns	34.75*
SN 3	4.23*	8.96*	9.71*	12.74*	29.40*	37.55ns
SN 5	0.86*	6.59*	8.08*	4.67*	20.12*	28.12*
SN 9	11.66*	23.82ns	7.59*	26.60*	52.00ns	28.21*
SN 11	0.97*	6.89*	7.70*	5.56*	21.68*	29.40*
SN 12	0.54*	30.55ns	6.03*	4.39*	63.19ns	18.81*
SN 13	1.30*	6.58*	6.40*	8.76*	22.10*	24.23*
SN 14	2.02*	14.07*	6.93*	10.63*	34.20ns	25.55*
SN 15	8.48*	29.65ns	9.04*	27.35*	55.25ns	34.86*
SN 18	5.37*	8.65*	7.71*	17.42*	23.82*	27.37*
SN 20	9.37*	37.67ns	6.34*	24.66*	69.93ns	20.28*
SN 25	1.47*	16.56*	8.59*	7.78*	39.39ns	27.52*
SN 28	3.56*	2.81*	7.67*	13.84*	13.92*	26.89*
SN 32	5.09*	5.37*	5.99*	17.09*	18.32*	20.88*
SN 40	1.11*	12.27*	8.91*	7.36*	33.88ns	33.34*
SN 51	3.71*	8.84*	7.37*	15.49*	27.02*	27.82*
SN 57	0.84*	10.15*	5.75*	3.88*	30.34*	20.06*
SN 58	0.93*	9.82*	8.15*	5.28*	28.03*	28.51*
SN 59	0.50*	13.20*	6.93*	3.31*	40.06ns	24.60*
SN 60	0.62*	11.76*	4.65*	3.79*	37.01ns	14.87*
Inpari 42 Agritan GSR	1.42*	6.39*	10.65ns	9.04*	20.32*	37.01*
Inpari 18	8.72*	19.00*	12.37ns	15.91*	40.86ns	39.32ns
TN 1 (Susceptible check)	35.97	38.93	18.34	66.12	48.57	57.32
Conde (Resistant check)	3.14*	2.23*	0.86*	12.73*	10.26*	5.89*
Mean	4.72	14.68	7.94	13.96	34.00	28.05

Note: P = Pathotype, * = Significantly different, ns = Not significantly different to susceptible check (TN-1) based on t-Dunnett's test at 0.05 level

42 Agritan GSR. Two lines (SN 28 and 32) or 10% were resistant to BLB disease pathotype IV in the generative phase, comparable to the Conde variety as tolerant variety check (Table 8).

Resistance to BLB pathotype VIII was evaluated on a scale of 1 and 3, with the criteria ranging from moderately resistant to resistant. A total of 16 lines (80%) were moderately resistant, and 4 lines (SN 12, 32, 57, and 60) were resistant to BLB disease pathotype VIII in the generative phase. The resistance of the 16 lines was similar to that of the parent Inpari 42 Agritan GSR, with the criteria of moderately resistant. The commercial variety Inpari 18 and the susceptible check variety TN-1 were classified as moderately susceptible (Table 8).

This research aligns with Putri et al. (2023), who identified the TN-1 variety as a differential variety susceptible to BLB pathotypes III, IV, and VIII, while the resistant check variety Conde was very resistant to these pathotypes in both growth phases. The Conde variety carries 2 BLB resistance genes (Xa4, Xa7) in all 3 pathotypes (Yuriyah et al., 2021). According to Noer et al. (2018), differential varieties carrying the Xa2, Xa4, and Xa21 genes were more resistant to the attacks by pathotypes I, II, III, V, and VI. Bakade et al. (2021) highlighted that there are many proteins associated with resistance to BLB disease, such as transmembrane leucine-rich repeat (LRR) protein kinase, serine/threonine protein kinase, protein kinase family genes, wall-associated kinase (WAK) which regulate transcription factors such as WRKY, bZIP, DOF, MYB, and HSFs. The main virulence of the *Xoo* pathogen includes exo-enzymes, exopolysaccharide (EPS), and resistant biofilm formation, which causes the development of BLB disease (Vishakha et al., 2020).

As illustrated in Figure 1, the leaf tips of each line have a shorter lesion length than the susceptible check TN-1. Although no highly resistant genotypes were identified, almost all genotypes had better resistance than the TN-1

Table 8. Score and criteria for BLB disease resistance in DH GSR lines in the generative phase

Genotype	Pathotype III		Pathotype IV		Pathotype VIII	
	Score	Criteria	Score	Criteria	Score	Criteria
SN 2	1	R	5	MS	3	MR
SN 3	1	R	3	MR	3	MR
SN 5	1	R	3	MR	3	MR
SN 9	3	MR	5	MS	3	MR
SN 11	1	R	3	MR	3	MR
SN 12	1	R	7	S	1	R
SN 13	1	R	3	MR	3	MR
SN 14	1	R	5	MS	3	MR
SN 15	3	MR	7	S	3	MR
SN 18	1	R	3	MR	3	MR
SN 20	3	MR	7	S	3	MR
SN 25	1	R	5	MS	3	MR
SN 28	1	R	1	R	3	MR
SN 32	1	R	1	R	1	R
SN 40	1	R	5	MS	3	MR
SN 51	1	R	3	MR	3	MR
SN 57	1	R	3	MR	1	R
SN 58	1	R	3	MR	3	MR
SN 59	1	R	5	MS	3	MR
SN 60	1	R	3	MR	1	R
Inpari 42 Agritan GSR	1	R	3	MR	3	MR
Inpari 18	3	MR	5	MS	5	MS
TN 1 (Susceptible chek)	7	S	7	S	5	MS
Conde (Resistance check)	1	R	1	R	1	R

Note: R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible

susceptible check against BLB pathotype VIII disease in the generative phase. *Xoo*-infected rice leaves show a distinct green coloration, which is associated with chlorophyll content and its role in photosynthesis (Cao et al., 2022). Resistance to BLB can also be influenced by leaf morphology in each genotype. According to Cao et al. (2020), *Xoo* enters through hydathodes or wounds on leaves and then reproduces in the xylem vessels, leading to BLB disease. Furthermore, Nurhayatini et al. (2020) found that a hairy leaf surface morphology is positively correlated with BLB resistance. Ahmad et al. (2023) noted that environmental factors, such as minimum temperature, atmospheric pressure, humidity, elevation, soil organic carbon, and pH, significantly influence the spread of BLB disease, as determined by spatial OLS regression models.

As depicted in Table 5 and 8, the resistance of the 20 tested lines varied across different BLB pathotypes and growth phases. For pathotype III, 8 lines (40%) were resistant in the vegetative phase, while 17 lines (85%) were resistant in the generative phase. For pathotype IV, 7 lines (35%) exhibited resistance in the vegetative phase, and 2 lines (10%) were resistant in the generative phase. For pathotype VIII, 11 lines (55%) were moderately resistant in the vegetative phase, and 16 lines (80%) were moderately resistant in the generative phase. Overall, the tested lines showed good resistance to BLB attacks, with the highest resistance observed to pathotype III, followed by pathotype IV and then pathotype VIII in the vegetative phase (pathotype III > IV > VIII vegetative phase). The lines generally displayed better resistance in the generative phase compared to the vegetative phase.

Out of the 20 lines tested, 4 lines (20%), namely SN 11, 13, 57, and 58 demonstrated good resistance, with the criteria being moderately resistant to resistant for the 3 pathotypes in both vegetative and generative phases (Table 5 and 8). These lines are recommended as new GSR lines. As stated in the standard for releasing food plant varieties issued by the Ministry of Agriculture of Indonesia (2021), Ministry of Agriculture of Indonesia (2021), a candidate variety must be at least moderately resistant to 1 of the BLB pathotypes. This research indicates that the tested lines not only meet but exceed the minimum BLB resistance.

Figure 1. Variation of lesion length in DH GSR lines against BLB pathotype VIII disease in the generative phase (left to right: Inpari 18, Inpari 42 Agritan GSR, 20 test lines, TN-1 susceptible check, and Conde resistant check)

The genes related to the virulence of the *Xoo* pathogen are very complex. *Xanthomonas oryzae* pv. *oryzae* is regulated by the RpoN2-PilRX regulatory system. The system controls transcription of the type IV pilus (T4P) gene, which is required in motility, surface adhesion, biofilm formation, and virulence in pathogenic bacteria (Yu et al., 2020). *Xoo* attacks rice plants using T3S effectors (T3SEs), namely transcription activator-like effectors (TALEs), which enter the nucleus (Ji et al., 2018). *Xoo* uses TALEs to bind effector binding elements (EBEs) in host plants, thereby inducing susceptibility genes in the host (OsSULTR3;6, OsSWEET11, and OsSWEET14) (Ni et al., 2021).

Resistance and susceptibility of rice to bacterial blight disease are controlled by molecular interactions between *Xoo* effectors and target genes in rice (Ji et al., 2018). Plants exposed to nitrogen allow *Xoo* to modulate fluxes through gluconeogenesis, glycogen biosynthesis, and degradation pathways (Koduru et al., 2020). Respiratory burst oxidase homologs (Rbohs) are critical enzymes in the generation of reactive oxygen species (ROS) enzymes that form ROS, where ROS functions as a signal in regulating plant development and stress (Zhu et al., 2024). Type III secretion systems (T3SS), namely XopN, XopQ, XopX, and XopZ, are the effectors used by *Xoo* to suppress rice immunity (Deb et al., 2022). Nucleotide-binding site–leucine-rich repeat (NB-LRR) resistance proteins that express *Oryza sativa* RPM1-like resistance gene 1 (OsRLR1) overexpression increases resistance to *Xoo* pathogens. The transcription factor OsWRKY19 also contributes to defense by binding to the OsPR10 promoter, activating pathogen defense mechanisms (Du et al., 2021).

Immune responses in plants can be mediated through 2 mechanisms: activating innate immunity and eliminating host susceptibility through interactions with effectors. Plants detect pathogens via innate immunity, which includes PAMPs-triggered immunity (PTI). This defense mechanism relies on pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) that enter the host plant's apoplast. Receptor kinases (RKs), as key components of PRRs, provide initial signals of pathogen attack. PRRs and PAMPs together trigger PTI to fight pathogens. Eight genes (Xa1, xa5, Xa10, xa13, Xa23, xa25, Xa27, xa41) mediate TALE-associated resistance to the pathogen, and 3 genes (Xa3/Xa26, Xa4, and Xa21) encode protein kinases and mediate rice resistance (Ji et al., 2018).

CONCLUSIONS

The interaction between genotype and pathotype affects disease severity and severity index in both growth phases. The lines tested have varying resistance levels from susceptible to resistant to BLB. Six lines (SN 11, 12, 13, 57, 58, and 59) were categorized as moderately resistant to resistant to 3 BLB pathotypes in the vegetative phase. Ten lines (SN 3, 5, 11, 13, 18, 28, 32, 57, 58, and 60) were moderately resistant to resistant to 3 BLB pathotypes in the generative phase. Four lines (SN 11, 13, 57, and 58) belonged to moderately resistant to resistant criteria against all 3 pathotypes in both growth phases. The selected lines can be used as a source of parents for breeders and candidates for new superior varieties with BLB resistance properties to support the reduction of synthetic chemical bactericide inputs and control BLB disease. However, these lines should be further evaluated for their performance in the field.

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REFERENCES

Agrios, G. N. (2005). *Plant pathology* (5th Edition). Burlington, USA: Elsevier Academic Press. https://doi.org/10.1016/C2009-0-02037-6

Ahmad, T. M., Haider, M. S., Randhir, T. O., Randhir, R., & Ahmad, S. R. (2023). Spatial analysis of factors influencing bacterial leaf blight in rice production. *Brazilian Journal of Biology*, *83*(e264249), 1–12. https://doi.org/10.1590/1519-6984.264249

Ahmed, T., Noman, M., Jiang, H., Shahid, M., Ma, C., Wu, Z., … & Li, B. (2022). Bioengineered chitosan-iron nanocomposite controls bacterial leaf blight disease by modulating plant defense response and nutritional status of rice (*Oryza sativa* L.). *Nano Today*, *45*, 101547. https://doi.org/10.1016/j.nantod.2022.101547

Ali, J., Nicolas, K. L. C., Akther, S., Torabi, A., Ebadi, A. A., Marfori-Nazarea, C. M., & Mahender, A. (2021). Improved anther culture media for enhanced callus formation and plant regeneration in rice (*Oryza sativa* L.). *Plants*, *10*(839), 1–16. https://doi.org/10.3390/plants10050839

Amanat, M. A., Naeem, M. K., Algwaiz, H. I. M., Uzair, M., Attia, K. A., Alkathani, M. D. F., … & Khan, M. R. (2022). Evaluation of green super rice lines for agronomic and physiological traits under salinity stress. *Plants*, *11*(11), 1461. https://doi.org/10.3390/plants11111461

Arshad, H. M. I., Saleem, K., Khan, J. A., Rashid, M., Atiq, M., Alam, S. S., & Sahi, S. T. (2017). Pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* isolates collected from Punjab Province of Pakistan. *European Journal of Plant Pathology*, *147*, 639–651. https://doi.org/10.1007/s10658-016-1032-5

Ashwini, D., Yadla, H., Padmaja, G., Seetalam, M., Hari, Y., Padmaja, G., Malathi, S., & Rao, P. J. M. (2021). Phenotypic and genotypic screening of certain rice genotypes against bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) resistance. *Indian Journal of Plant Protection*, *49*(3), 190–195. Retrieved from https://epubs.icar.org.in/index.php/IJPP/article/view/119344

Asysyuura, Nawangsih, A. A., Mutaqin, K. H., & Sudir. (2017). Pathotype identification of *Xanthomonas oryzae* pv. *oryzae* isolates from South Sulawesi. *Jurnal Fitopatologi Indonesia*, *13*(3), 73–80. https://doi.org/10.14692/jfi.13.3.73

Azizi, M. M. F., & Lau, H. Y. (2022). Advanced diagnostic approaches developed for the global menace of rice diseases: A review. *Canadian Journal of Plant Pathology*, *44*(5), 627–651. https://doi.org/10.1080/07060661.2022.2053588

Bakade, R., Ingole, K. D., Deshpande, S., Pal, G., Patil, S. S., Bhattacharjee, S., Prasannakumar, M. K., & Ramu, V. S. (2021). Comparative transcriptome analysis of rice resistant and susceptible genotypes to *Xanthomonas oryzae* pv. *oryzae* identifies novel genes to control bacterial leaf blight. *Molecular Biotechnology*, *63*, 719–731. https://doi.org/10.1007/s12033-021-00338-3

Biswas, P. L., Nath, U. K., Ghosal, S., Goswami, G., Uddin, M. S., Ali, O. M., … & Hossain, A. (2021). Introgression of bacterial blight resistance genes in the rice cultivar Ciherang: Response against *Xanthomonas oryzae* pv. *oryzae* in the F6 generation. *Plants*, *10*(10), 2048. https://doi.org/10.3390/plants10102048

Bock, C. H., Barbedo, J. G. A., Del Ponte, E. M., Bohnenkamp, D., & Mahlein, A.-K. (2020). From visual estimates to fully automated sensor-based measurements of plant disease severity: Status and challenges for improving accuracy. *Phytopathology Research*, *2*, 9. https://doi.org/10.1186/s42483-020-00049-8

Cao, J., Chu, C., Zhang, M., He, L., Qin, L., Li, X., & Yuan, M. (2020). Different cell wall-degradation ability leads to tissue-specificity between *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola*. *Pathogens*, *9*(3), 187. https://doi.org/10.3390/pathogens9030187

Cao, Y., Xu, H., Song, J., Yang, Y., Hu, X., Wiyao, K. T., & Zhai, Z. (2022). Applying spectral fractal dimension index to predict the

SPAD value of rice leaves under bacterial blight disease stress. *Plant Methods*, *18*(1), 67. https://doi.org/10.1186/s13007-022-00898-8

Chen, S., Wang, C., Su, J., Feng, A., Zhu, X., & Zeng, L. (2018). Differential detection and analysis of pathotypes and differentiation against *Xanthomonas oryzae* pv. *oryzae* in Southern China. *Agricultural Biotechnology*, *7*(1), 92–96. Retrieved from https://www.proquest.com/scholarly-journals/differential-detection-analysis-pathotypes/docview/2135998497/se-2

Chukwu, S. C., Rafii, M. Y., Ramlee, S. I., Ismail, S. I., Hasan, M. M., Oladosu, Y. A., … & Olalekan, K. K. (2019). Bacterial leaf blight resistance in rice: A review of conventional breeding to molecular approach. *Molecular Biology Reports*, *46*(1), 1519–1532. https://doi.org/10.1007/s11033-019-04584-2

Deb, S., Gokulan, C. G., Nathawat, R., Patel, H. K., & Sonti, R. V. (2022). Suppression of XopQ–XopX-induced immune responses of rice by the type III effector XopG. *Molecular Plant Pathology*, *23*(5), 634–648. https://doi.org/10.1111/mpp.13184

Deng, W. L., Lin, H. A., Shih, Y. C., Kuo, C. C., Tzeng, J. Y., Liu, L. Yu D., Huang, S. T., Huang, C. M., & Chung, C. L. (2016). Genotypic and pathotypic diversity of *Xanthomonas oryzae* pv. *oryzae* strains in Taiwan. *Journal of Phytopathology*, *164*(10), 745–759. https://doi.org/10.1111/jph.12495

Ding, X., Huang, X., Sun, L., Wu, J., & Liu, J. (2019). Influence of abscisic acid-biosynthesis inhibitor fluridone on the feeding behavior and fecundity of *Nilaparvata lugens*. *Insects*, *10*(2), 57. https://doi.org/10.3390/insects10020057

Du, D., Zhang, C., Xing, Y., Lu, X., Cai, L., Yun, H., … & He, G. (2021). The CC-NB-LRR OsRLR1 mediates rice disease resistance through interaction with OsWRKY19. *Plant Biotechnology Journal*, *19*(5), 1052–1064. https://doi.org/10.1111/pbi.13530

Duy, P. N., Lan, D. T., Thu, H. P., Thu, H. P. T., Thanh, H. N., Pham, N. P., … & Pham, X. H. (2021). Improved bacterial leaf blight disease resistance in the major elite Vietnamese rice cultivar TBR225 via editing of the OsSWEET14 promoter. *PLoS ONE*, *16*(9), e0255470. https://doi.org/10.1371/journal.pone.0255470

Habib, A., Abdullah, A., & Puyam, A. (2022). Visual estimation: A classical approach for plant disease estimation. *Trends in plant disease assessment*, pp. 19–45. Singapore: Springer. https://doi.org/10.1007/978-981-19-5896-0_2

Hamid, M. I., & Ghazanfar, M. U. (2022). Integrated management of rice diseases. *Modern techniques of rice crop production* (pp. 401–421). Springer. https://doi.org/10.1007/978-981-16-4955-4_22

Huerta, A. I., Delorean, E. E., Bossa-Castro, A. M., Tonnessen, B. W., Raghavan, C., Corral, R., … & Leach, J. E. (2021). Resistance and susceptibility QTL identified in a rice MAGIC population by screening with a minor-effect virulence factor from *Xanthomonas oryzae* pv. *oryzae*. *Plant Biotechnology Journal*, *19*(1), 51–63. https://doi.org/10.1111/pbi.13438

IRRI. (2014). *Standard evaluation system (SES) for rice* (5th ed.). Manila, Philippines: International Rice Research Institute. Retrieved from https://www.scribd.com/document/333585255/SES-5th-Edition

Jewel, Z. A., Ali, J., Pang, Y., Mahender, A., Acero, B., Hernandez, J., Xu, J., & Li, Z. (2019). Developing green super rice varieties with high nutrient use efficiency by phenotypic selection under varied nutrient conditions. *The Crop Journal*, *7*(3), 368–377. https://doi.org/10.1016/j.cj.2019.01.002

Ji, Z., Wang, C., & Zhao, K. (2018). Rice routes of countering *Xanthomonas oryzae*. *International Journal of Molecular Sciences*, *19*(10), 3008. https://doi.org/10.3390/ijms19103008

Jiang, N., Yan, J., Liang, Y., Shi, Y., He, Z., Wu, Y., Zeng, Q., Liu, X., & Peng, J. (2020). Resistance genes and their interactions with bacterial blight/leaf streak pathogens (*Xanthomonas oryzae*) in rice (*Oryza sativa* L.)—an updated review. *Rice*, *13*(3), 1–12. https://doi.org/10.1186/s12284-019-0358-y

Khannetah, K. R., Ramchander, S., Leon, M. T. A. P., Shoba, D., Saravanan, S., Kannan, R., Yasin, J. K., & Pillai, M. A. (2021). Genetic diversity analysis in indigenous rice (*Oryza sativa* L.) germplasm for bacterial leaf blight

(*Xanthomonas oryzae* pv. *oryzae*) (BB) using resistance genes-linked markers. *Euphytica*, *217*(7), 145. https://doi.org/10.1007/s10681-021-02862-4

Koduru, L., Kim, H. Y., Lakshmanan, M., Mohanty, B., Lee, Y. Q., Lee, C. H., & Lee, D. Y. (2020). Genome-scale metabolic reconstruction and in silico analysis of the rice leaf blight pathogen, *Xanthomonas oryzae*. *Molecular Plant Pathology*, *21*(4), 527–540. https://doi.org/10.1111/mpp.12914

Li, Z., & Ali, J. (2017). Breeding green super rice (GSR) varieties for sustainable rice cultivation. *Achieving sustainable cultivation of rice* (pp. 109–129). https://doi.org/10.19103/as.2016.0003.05

Liu, W., Liu, J., Triplett, L., Leach, J. E., & Wang, G. L. (2014). Novel insights into rice innate immunity against bacterial and fungal pathogens. *Annual Review of Phytopathology*, *52*, 213–241. https://doi.org/10.1146/annurev-phyto-102313-045926

Ministry of Agriculture of Indonesia. (2021). *Standar operasional prosedur penilaian calon varietas dalam rangka pelepasan varietas tanaman pangan.* Jakarta: Ministry of Agriculture of Indonesia.

Ni, Z., Cao, Y., Jin, X., Fu, Z., Li, J., Mo, X., He, Y., Tang, J., & Huang, S. (2021). Engineering resistance to bacterial blight and bacterial leaf streak in rice. *Rice*, *14*(38), 1–5. https://doi.org/10.1186/s12284-021-00482-z

Noer, Z., Hasanuddin, Lisnawita, & Suryanto, D. (2018). Pathotype profile of *Xanthomonas oryzae* pv. *oryzae* isolates from North Sumatera. *IOP Conference Series: Earth and Environmental Science*, *122*(1), 012142. https://doi.org/10.1088/1755-1315/122/1/012142

Nurhayatini, R., Budiasih, R., Amalia, L., Parlinah, L., & Suryana, Y. (2020). Effect of Corynebacterium against *Xanthomonas campestris* causes of bacterial leaf blight in the paddy plant (*Oryza sativa* L.) varieties Inpari 13. *Proceedings of the International Conference on Agriculture, Social Sciences, Education, Technology and Health (ICASSETH 2019)* (pp. 115–117). Atlantis Press. https://doi.org/10.2991/assehr.k.200402.026

Nurhidayah, S., Purwoko, B. S., Dewi, I. S., Suwarno, W. B., & Lubis, I. (2023). Agronomic performance and selection of green super rice doubled haploid lines from anther culture. *Biodiversitas*, *24*(2), 819–826. https://doi.org/10.13057/biodiv/d240218

Pradhan, S. K., Nayak, D. K., Mohanty, S., Behera, L., Barik, S. R., Pandit, E., Lenka, S., & Anandan, A. (2015). Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. *Rice*, *8*(19), 1–14. https://doi.org/10.1186/s12284-015-0051-8

Putri, R. K., Purwoko, B. S., Dewi, I. S., Lubis, I., & Yuriyah, S. (2023). Resistance of doubled haploid rice lines to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*). *SABRAO Journal of Breeding and Genetics*, *55*(3), 717–728. https://doi.org/10.54910/sabrao2023.55.3.10

Quibod, I. L., Atieza-Grande, G., Oreiro, E. G., Palmos, D., Nguyen, M. H., Coronejo, S. T., … & Oliva, R. (2020). The green revolution shaped the population structure of the rice pathogen *Xanthomonas oryzae* pv. *oryzae*. *ISME Journal*, *14*(2), 492–505. https://doi.org/10.1038/s41396-019-0545-2

Rachman, B., Ariningsih, E., Sudaryanto, T., Ariani, M., Septanti, K. S., Adawiyah, C. R., … & Yuniarti, E. (2022). Sustainability status, sensitive and key factors for increasing rice production: A case study in West Java, Indonesia. *PLoS ONE*, *17*(12), e0274689. https://doi.org/10.1371/journal.pone.0274689

Sanya, D. R. A., Syed-Ab-Rahman, S. F., Jia, A., Onésime, D., Kim, K.-M., Ahohuendo, B. C., & Rohr, J. R. (2022). A review of approaches to control bacterial leaf blight in rice. *Journal of Microbiology and Biotechnology*, *38*(7), 113. https://doi.org/10.1007/s11274-022-03298-1

Saputra, M. H., Sutomo, Humaida, N., & Hadiyan, Y. (2023). Smart farming: Modeling distribution of *Xanthomonas campestris* pv. *oryzae* as a leaf blight-causing bacteria in rice plants. *IOP Conference Series: Earth and Environmental Science*, *1133*(1), 012026. https://doi.org/10.1088/1755-1315/1133/1/012026

Sastro, Y., Suprihanto, Hairmansis, A., Hasmi, I., Rumanti, I. A., Susanti, Z., … & Arismiati,

D. (2021). *Deskripsi varietas unggul baru padi*. Sukamandi, Indonesia: Agricultural Research and Development Center, Ministry of Agriculture. Retrieved from https://www.researchgate.net/publication/356696369

Singh, B. K., Delgado-Baquerizo, M., Egidi, E., Guirado, E., Leach, J. E., Liu, H., & Trivedi, P. (2023). Climate change impacts on plant pathogens, food security and paths forward. *Nature Reviews Microbiology*, *21*(10), 640–656. https://doi.org/10.1038/s41579-023-00900-7

Statistics Indonesia. (2024a). *Luas panen, produksi, dan produktivitas padi menurut provinsi*. Retrieved from https://www.bps.go.id/id/statistics-table/2/MTQ5OCMy/luas-panen--produksi--dan-produktivitas-padi-menurut-provinsi.html

Statistics Indonesia. (2024b). *Impor beras menurut negara asal utama, 2017-2023*. Retrieved from https://www.bps.go.id/id/statistics-table/1/MTA0MyMx/impor-beras-menurut-negara-asal-utama--2000-2022.html

Subehi, M., Abdurachman, A. A., Hasanah, L., Gultom, R., Jaka, S., Uliyah, Sulistiyowati, H., Martono, H. D., Heruwaty, & Indah, K. (2020). *Statistics of climate, crop pest and climate change impact 2017-2020*. Center for Agricultural Data and Information System, Secretariate General, Ministry of Agriculture. Retrieved from https://satudata.pertanian.go.id/assets/docs/publikasi/Statistik_Iklim,_OPT_dan_DPI_2017-2020.pdf

Sudir, & Yuliani, D. (2016). Composition and distribution of *Xanthomonas oryzae* pv. *oryzae* pathotypes, the pathogen of rice bacterial leaf blight in Indonesia. *Agrivita*, *38*(2), 174–185. https://doi.org/10.17503/agrivita.v38i2.588

Susanto, U., Gumelar, A. I., & Ali, J. (2022). Genetic variability and heritability of agronomic traits of selected green super rice (GSR) lines in Indonesia. *IOP Conference Series: Earth and Environmental Science*, *978*(1), 012014. https://doi.org/10.1088/1755-1315/978/1/012014

Tudi, M., Ruan, H. D., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T. (2021). Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health*, *18*(3), 1112. https://doi.org/10.3390/ijerph18031112

Vishakha, K., Das, S., Banerjee, S., Mondal, S., & Ganguli, A. (2020). Allelochemical catechol comprehensively impedes bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*. *Microbial Pathogenesis*, *149*, 104559. https://doi.org/10.1016/j.micpath.2020.104559

Wijayanto, A. K., Prasetyo, L. B., Hudjimartsu, S. A., Sigit, G., & Hongo, C. (2024). Textural features for BLB disease damage assessment in paddy fields using drone data and machine learning: Enhancing disease detection accuracy. *Smart Agricultural Technology*, *8*, 100498. https://doi.org/10.1016/j.atech.2024.100498

Yasmin, S., Zaka, A., Imran, A., Zahid, M. A., Yousaf, S., Rasul, G., Arif, M., & Mirza, M. S. (2016). Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. *PLoS ONE*, *11*(8), e0160688. https://doi.org/10.1371/journal.pone.0160688

Yu, C., Nguyen, D. P., Ren, Z., Liu, J., Yang, F., Tian, F., Fan, S., & Chen, H. (2020). The RpoN2-PilRX regulatory system governs type IV pilus gene transcription and is required for bacterial motility and virulence in *Xanthomonas oryzae* pv. *oryzae*. *Molecular Plant Pathology*, *21*(5), 652–666. https://doi.org/10.1111/mpp.12920

Yugander, A., Sundaram, R. M., & Ladhalakshmi, D. (2017). Virulence profiling of *Xanthomonas oryzae* pv. *oryzae* isolates, causing bacterial blight of rice in India. *European Journal of Plant Pathology*, *149*, 171–191. https://doi.org/10.1007/s10658-017-1176-y

Yuriyah, S., Darnaedi, D., Setia, T. M., Windarsih, G., & Utami, D. W. (2021). Phenotype and genotype variability of interspecific rice lines related to bacterial leaf blight resistance (*Xanthomonas oryzae* pv. *oryzae*) character. *Biodiversitas*, *22*(10), 4123–4130. https://doi.org/10.13057/biodiv/d221001

Zafar, K., Khan, M. Z., Amin, I., Mukhtar, Z., Yasmin, S., Arif, M., Ejaz, K., & Mansoor, S. (2020). Precise CRISPR-Cas9 mediated genome editing in super basmati rice for

resistance against bacterial blight by targeting the major susceptibility gene. *Frontiers in Plant Science*, *11*, 532184. https://doi.org/10.3389/fpls.2020.00575

Zafar, S. A., Arif, M. H., Uzair, M., Rashid, U., Naeem, M. K., Rehman, O. U., … & Khan, M. R. (2022). Agronomic and physiological indices for reproductive stage heat stress tolerance in green super rice. *Agronomy*, *12*(8), 1907. https://doi.org/10.3390/agronomy12081907

Zhu, Y., Su, H., Liu, X. X., Sun, J. F., Xiang, L., Liu, Y. J., … & Li, Y. (2024). Identification of NADPH oxidase genes crucial for rice multiple disease resistance and yield traits. *Rice*, *17*(1), 1–15. https://doi.org/10.1186/s12284-023-00678-5