RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
55 (3) 717-728, 2023
http://doi.org/10.54910/sabrao2023.55.3.10
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

RESISTANCE OF DOUBLED HAPLOID RICE LINES TO BACTERIAL LEAF BLIGHT (*Xanthomonas oryzae* pv. *oryzae*)

R.K. PUTRI[1], B.S. PURWOKO[2*], I.S. DEWI[3], I. LUBIS[2], and S. YURIYAH[3]

[1]Study Program of Plant Breeding and Biotechnology, the Graduate School, IPB University, Bogor, Indonesia
[2]Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor, Indonesia
[3]Research Organization of Agriculture and Food, National Research and Innovation Agency (BRIN), Bogor, Indonesia
*Corresponding author's email: bspurwoko@apps.ipb.ac.id
Email addresses of co-authors: ratnakartika@apps.ipb.ac.id, iswari.dewi01@gmail.com, iskandarlbs@yahoo.com, sitiyur@yahoo.co.id

SUMMARY

Developing new high-yielding rice varieties resistant to bacterial leaf blight (BLB) is an effective strategy for controlling BLB. Several advanced doubled haploid rice lines derived from anther culture previously selected need assessment for BLB resistance. This study aimed to evaluate the resistance of these lines to BLB pathotypes III, IV, and VIII in the vegetative and generative phases. The experiment took place in a greenhouse using 16 rice genotypes comprising 12 doubled haploid rice lines, two commercial check varieties (Inpari 18 and Inpari 34), and a BLB-resistant and susceptible check variety (Code and TN-1, respectively). Inoculation began with the leaf clipping method using a suspension of the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) at a concentration of 10^9 cfu/ml. The results indicated significant influences on disease severity and intensity of BLB of pathotype, genotype, and the interactions between pathotype and genotype, finding their values higher in the vegetative phase. Six doubled haploid lines ranged from resistant to moderately resistant (disease severity 2.0%–10.7%, disease intensity 6.7%–36.8%) to pathotypes III and IV in two growth phases, i.e., HS1-35-1-4, HS4-15-1-9, HS4-15-1-16, HS4-15-1-24, HS4-15-1-26, and HS4-15-1-28. All those doubled haploid lines were susceptible to BLB pathotype VIII in the vegetative phase and moderately susceptible in the generative phase.

Keywords: Bacterial leaf blight, disease intensity, disease severity, doubled haploid rice lines

Key findings: The pathotype, genotype, and interactions between pathotype and genotype significantly affected the severity and intensity of BLB. The genotype resistance varied. Six doubled haploid lines exhibited moderate resistant to resistant to BLB pathotypes III and IV. The result of this study is crucial for use in consideration of variety release.

Communicating Editor: Dr. Aris Hairmansis

Citation: Putri RK, Purwoko BS, Dewi IS, Lubis I, Yuriyah S (2023). Resistance of doubled haploid rice lines to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*). *SABRAO J. Breed. Genet.* 55(3): 717-728. http://doi.org/10.54910/sabrao2023.55.3.10.

Manuscript received: March 4, 2023; Accepted: May 29, 2023.

INTRODUCTION

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most critical diseases that caused yield losses of more than 50% in rice plants (Yasmin *et al.*, 2017). High yield losses may occur depending on the type of variety used, plant growth phases, geographical location, and environmental conditions (Liu *et al.*, 2014). BLB infects rice crops widely in Australia, America, West Africa, and Asia, including India, the Philippines, Nepal, Sri Lanka, and Indonesia (Naqvi, 2019). The BBPOPT (2021) reported that in 2021, BLB affected about 32,691 ha of Indonesian rice production area, which increased from 27,958 ha in 2017. The most severe BLB attacks on rice cultivation in Indonesia reported in 2010, affected 40,486 ha in West Java, 30,029 ha in Central Java, 23,504 ha in East Java, 3,745 ha in Banten, and 2,678 ha in Southeast Sulawesi (Ditlin, 2011). Infection with BLB can occur in all phases of rice growth through the leaf epidermis pore (hydathodes) or wounds. Then the pathogen multiplies within cells (intracellular structures), infects the vascular tissues and is distributed to plants (Lee *et al.*, 2011). Symptoms that first appear are yellow to white stripes on the edges of the leaves, expanding to a grayish color, causing the leaves to turn yellow and dry (blight phase), then wilting as the most damaging phase causing the death of the leaves (The "kresek" phase) (Ou, 1985).

Using varieties resistant to various *Xoo* pathotypes is the most economical and effective strategy for controlling BLB disease, playing a crucial role in sustaining rice productivity that requires no additional cost to farmers and is environmentally safe (Fatimah *et al*., 2019). The current 11 *Xoo* pathotypes identified in Indonesia had three of them (Pathotypes III, IV, and VIII) as dominant (Sudir and Yuliani, 2016). Pathotype III thrives in South Sulawesi, Kalimantan, Java, and Bali; Pathotype IV (extra virulent) prevails in Java, Bali, and South Sulawesi, while Pathotype VIII was dominant in West Java. Wang *et al.* (2020) reported that of the 45 BLB resistance genes identified, 11 faced successful cloning and characterization, including *Xa1*, *Xa4*, *xa5*, *Xa10*, *xa13*, *Xa21*, *Xa23*, *xa25*, *Xa3/Xa26*, *xa27*, and *xa41* (Ji *et al.*, 2018). Some of them have headed for high yielding BLB-resistant variety development.

Resistance evaluation of rice genotypes to BLB can be through phenotypic scoring based on the response of plant leaves that *Xoo* infects. Khaeruni *et al.* (2016) revealed that several local rice cultivars had varied resistance responses, ranging from susceptible to highly resistant (disease severity 2.6%–77.6%). Another research done by Herlina and Silitonga (2011) evaluated 150 rice varieties (ICABIOGRAD collection), resulting in varied Standard Evaluation System (SES) for Rice from IRRI (1996) scores ranging from one to seven (resistant to moderately susceptible). Acharya and Sujata (2021) also mentioned varied resistance for BLB, from resistant to highly susceptible, due to different genetic backgrounds of tested 150 rice genotypes in 2018 and 315 in 2019.

Resistance to BLB information is valuable for use in releasing new rice varieties. Several doubled haploid (DH) rice advanced lines derived from anther cultures of F_1 from crosses with superior parents has been selected (Safitri *et al.*, 2016; Anshori, 2018; Anshori *et al.*, 2019). Furthermore, these advanced lines need assessment to determine their resistance to BLB. Therefore, this study sought to evaluate the resistance of doubled haploid rice advanced lines to BLB pathotypes III, IV, and VIII.

MATERIALS AND METHODS

The genetic material used in this study were 16 rice genotypes consisting of 12 doubled haploid (DH) rice lines, two commercial check varieties (Inpari 18 and Inpari 34), and two checks for

Table 1. The DH rice lines used in the evaluation of BLB.

S. No.	Genotype	S.No.	Genotype	S.No.	Genotype
1	HS4-11-1-73	5	HS4-15-1-22	9	HS4-15-1-43
2	HS1-35-1-4	6	HS4-15-1-24	10	HS4-15-1-63
3	HS4-15-1-9	7	HS4-15-1-26	11	HS4-15-1-70
4	HS4-15-1-16	8	HS4-15-1-28	12	HS4-15-2-9

[1] Source: Anshori (2018); HS1=Inpara 5/IR77674; HS4=IR77674/Inpari 29; S.No.: serial number

Table 2. Criteria for BLB resistance based on disease severity[1].

Value Scale	Area symptom/Disease severity (%)	Level of resistance
0	0	HR= Highly resistant
1	1–6	R= Resistant
3	>6–12	MR= Moderately resistant
5	>12–25	MS= Moderately susceptible
7	>25–50	S= Susceptible
9	>50–100	HS= Highly susceptible

[1] Source: SES from IRRI (2014).

BLB resistance, namely, Code (resistant to BLB) and TN-1 (susceptible to BLB) (Table 1). The experiment was conducted in a greenhouse at the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) with three replications for each pathotype.

Sowing followed on a seedbed after germinating each genotype in a petri dish. Transplanting continued 14 days after sowing (DAS) in a pot (16 cm × 19 cm) filled with muddy soil. Each vessel consisted of five plants and had three replicates per pot. Planting each genotype in exact rows comprised two commercial check varieties, a cultivar resistant to BLB and one susceptible to BLB. Applying fertilizer had a rate of 1 g N + 1 g K_2O + 1 g P_2O_5 per pot. Additional fertilizer one and two months after planting had a rate of 2 g N per pot. The vegetative and generative tests used the same plants grown under similar greenhouse conditions.

Propagating the BLB pathotypes III, IV, and VIII isolates ensued on Wakimoto's medium. Screening for BLB resistance ran on two different plant growth phases, i.e., the vegetative and generative phases. The inoculation process started late afternoon to avoid scorching heat and high evaporation. The leaf clipping method, as described by Kauffman *et al*. (1973), proceeded for pathogen inoculation both in the vegetative (carried out at 35 DAT) and generative phases (carried out at 55–60 DAT), using scissors previously dipped in a suspension of the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) at a concentration of 10^9 cfu/ml, cut approximately 3 cm of leaf tips of fully developed leaves.

Observations began 14 days after inoculation (DAI) in the vegetative and generative phases by measuring the severity of the disease based on the lesion length on the leaf caused by BLB (cm) divided by total leaf length (cm) and multiplied by 100%. Then, converting the percentage according to the level of resistance scoring used the IRRI Standard Evaluation System for Rice (IRRI, 2014) (Table 2). Disease intensity calculation as disease severity index employed the formula, according to Yuliani *et al*. (2017):

$$DI = (\Sigma\ (ni \times vi)) / (Z \times N) \times 100\%$$

Where, DI = disease intensity (%), ni = number of samples with a certain severity score, vi = severity score (0–9), Z = the highest severity score, and N = total number of samples. The statistical analysis in this study used an analysis of variance and the least significant difference (LSD) test at the level of 5%.

RESULTS

Genotype resistance to pathotype III, IV, and VIII of BLB in the vegetative phase

Statistical analysis (Table 3) revealed that genotype, pathotype, and the interaction of genotype and pathotype significantly affected the severity and intensity of bacterial leaf blight in the vegetative phase. Pathotype contributes the most to the variation of disease severity and intensity, followed by genotype and the interaction between genotype and pathotype. The disease severity of *Xoo* pathotypes III, IV, and VIII varied from 4.7% to 70%, while disease intensity ranged from 13.0% to 92.2% (Table 4). Code (BLB-resistant check) consistently showed the lowest disease severity of all pathotypes, whereas TN-1 (BLB-susceptible check) showed the highest.

The disease severity of 12 DH lines against pathotypes III and IV was not significantly different from Code (BLB resistant check); yet, for pathotype VIII, all DH lines' disease severity revealed significantly different from and higher than Code, but still lower than TN-1. The disease severity of all genotypes (except TN-1) against pathotypes III and IV nonsignificantly differed, but both are significantly different from and lower than that of pathotype VIII. Thus, generally, pathotype VIII showed the highest disease severity compared to pathotypes III and IV for all genotypes in the vegetative phase.

Table 3. Analysis of variance for disease severity and intensity of BLB in the vegetative phase.

Traits	Mean Squares			
	Replication (df=2)	Genotypes (df=15)	Pathotypes (df=2)	Geno×Patho (df=30)
Disease severity	128.39813^{ns}	578.77916**	14281.02322**	186.16505**
Disease intensity	649.09521**	1373.67272**	29504.55250**	445.90206**

Table 4. Disease severity and intensity of all genotypes for BLB pathotypes III, IV, and VIII in the vegetative phase.

Genotypes	Disease Severity (%)			Disease Intensity (%)		
	P III	P IV	P VIII	P III	P IV	P VIII
HS4-11-1-73	4.0	8.9	55.5	13.0	33.3	92.2
HS1-35-1-4	5.9	9.6	36.5	18.5	30.4	77.8
HS4-15-1-9	8.4	6.6	42.9	24.4	22.2	80.0
HS4-15-1-16	7.9	7.2	45.5	30.4	29.4	84.4
HS4-15-1-22	5.4	10.5	53.3	17.0	34.8	85.2
HS4-15-1-24	7.9	8.9	45.9	26.3	29.6	81.5
HS4-15-1-26	8.6	11.9	38.1	32.6	35.9	76.3
HS4-15-1-28	10.7	9.4	40.6	36.8	34.8	76.3
HS4-15-1-43	9.8	13.0	44.4	33.3	45.2	86.7
HS4-15-1-63	11.7	14.9	44.9	36.3	35.2	85.2
HS4-15-1-70	18.9	7.8	37.5	55.6	28.9	76.3
HS4-15-2-9	12.4	8.3	37.4	39.3	33.3	79.3
Inpari 18	11.7	9.6	22.0	25.6	17.0	34.8
Inpari 34	6.1	6.6	26.4	23.0	27.4	67.4
Code	5.5	4.7	5.3	18.0	17.8	17.0
TN-1	20.3	44.5	70.0	48.9	80.7	92.2
LSD α= 0.05	13.25			17.06		

The disease intensity caused by pathotype VIII was the highest and significantly higher than pathotypes III and IV (except Code and Inpari 18) (Table 4). The disease intensity between pathotypes III and IV was unremarkably different in 12 of the 16 genotypes tested. Code had the lowest disease intensity compared to other genotypes for all three pathotypes. There were 10 DH lines against pathotype III and 11 DH lines against pathotype IV having disease intensity equivalent to Code; however, against pathotype VIII, the disease severity of all doubled haploid lines was significantly higher than Code and did not differ from that of TN-1 (BLB-susceptible check).

Screening for resistance among the genotypes tested in the vegetative phase ranged from highly susceptible to resistant. Among 16 genotypes tested, only Code was simultaneously resistant to three BLB pathotypes tested, namely, pathotypes III, IV, and VIII (Table 5). HS4-11-1-73, HS1-35-1-4, and HS4-15-1-22 were resistant to pathotype III (score of 1). Nine other genotypes were moderately resistant, with three moderately susceptible (HS4-15-1-70, HS4-15-2-9, and TN-1).

Screening for resistance to pathotype IV found that only Code was resistant. The 12 genotypes tested, including Inpari 18 and Inpari 34, emerged as moderately resistant with a score of three, while HS4-15-1-43 and HS4-15-1-63 were moderately susceptible, and TN-1 was susceptible. Almost all genotypes tested were categorically susceptible to and highly susceptible against pathotype VIII in the vegetative phase, except Code (resistant) and Inpari 18 (moderately susceptible). HS1-35-1-4 and HS4-15-1-22 had the best resistance score in the vegetative stage compared to other doubled haploid lines; both were resistant to pathotype III, moderately resistant to pathotype IV, and susceptible to pathotype VIII (Table 5).

Table 5. Results of genotype evaluation for resistance to BLB pathotypes III, IV, and VIII evaluations in the vegetative phase based on SES from IRRI (2014).

Genotypes	P III		P IV		P VIII	
	Score	Criteria	Score	Criteria	Score	Criteria
HS4-11-1-73	1	R	3	MR	9	HS
HS1-35-1-4	1	R	3	MR	7	S
HS4-15-1-9	3	MR	3	MR	7	S
HS4-15-1-16	3	MR	3	MR	7	S
HS4-15-1-22	1	R	3	MR	9	HS
HS4-15-1-24	3	MR	3	MR	7	S
HS4-15-1-26	3	MR	3	MR	7	S
HS4-15-1-28	3	MR	3	MR	7	S
HS4-15-1-43	3	MR	5	MS	7	S
HS4-15-1-63	3	MR	5	MS	7	S
HS4-15-1-70	5	MS	3	MR	7	S
HS4-15-2-9	5	MS	3	MR	7	S
Inpari 18	3	MR	3	MR	5	MS
Inpari 34	3	MR	3	MR	7	S
Code	1	R	1	R	1	R
TN-1	5	MS	7	S	9	HS

Note: P= pathotype; R= resistant; MR= moderately resistant; MS= moderately susceptible; S= susceptible; HS= highly susceptible.

Genotype resistance to pathotypes III, IV, and VIII of BLB in the generative phase

Based on the analysis of variance, the severity and intensity of BLB in the generative phase had pathotype mainly influencing it significantly, followed by genotype and the interaction between genotype and pathotype (Table 6). Generally, pathotypes IV and VIII showed higher disease severity and intensity than pathotype III in the generative phase. Based on the least significant difference (LSD) test, all DH lines against pathotype VIII showed significantly higher disease severity and intensity than pathotypes III and IV (Table 7).

Code had the lowest disease severity and intensity simultaneously to three pathotypes compared to other genotypes tested. Against pathotype III, all genotypes (except TN-1) had disease severity and intensity with no significant difference from Code. Disease severity and intensity of all DH lines differed nonsignificantly from commercial varieties tested, while nine did not vary considerably from Code in response to pathotype IV (Table 7). Responding to pathotype VIII, only Inpari 18 had disease severity and intensity that did not significantly differ from Code. The other genotypes showed high disease severity and intensity, with TN-1 having the highest disease severity and intensity value, especially for BLB pathotype IV.

Table 6. Analysis of variance for disease severity and intensity of BLB in the generative phase.

Traits	Mean Squares			
	Replication (df=2)	Genotypes (df=15)	Pathotypes (df=2)	Geno×Patho (df=30)
Disease severity	38.36583^{ns}	676.66266**	790.28771**	97.31741**
Disease intensity	269.05021^{ns}	1982.21778**	14218.31771**	503.09949**

Df= degree of freedom; ns= not significant at α= 0.05; **= significant at α= 0.01.

Table 7. Disease severity and intensity of all genotypes for BLB pathotypes III, IV, and VIII in the generative phase.

Genotypes	Disease Severity (%)			Disease Intensity (%)		
	P III	P IV	P VIII	P III	P IV	P VIII
HS4-11-1-73	2.6	6.3	15.1	10.7	23.0	52.6
HS1-35-1-4	3.6	5.4	15.8	11.4	20.7	51.9
HS4-15-1-9	4.1	4.8	18.4	17.0	15.6	57.7
HS4-15-1-16	2.7	3.6	14.2	8.0	11.1	50.0
HS4-15-1-22	1.7	3.6	12.7	7.2	11.1	43.7
HS4-15-1-24	2.0	10.7	15.9	6.7	35.2	54.1
HS4-15-1-26	2.9	5.9	14.4	9.6	19.0	87.2
HS4-15-1-28	2.7	9.3	15.4	9.6	34.3	52.6
HS4-15-1-43	3.4	8.5	14.4	12.6	33.3	51.1
HS4-15-1-63	3.4	4.9	15.8	9.6	20.0	50.1
HS4-15-1-70	5.0	5.6	13.2	20.0	20.0	46.3
HS4-15-2-9	4.1	4.1	15.2	15.6	14.1	49.6
Inpari 18	2.7	9.1	6.1	6.7	22.2	24.4
Inpari 34	3.1	9.8	10.6	11.1	33.3	43.0
Code	2.0	2.6	2.6	10.6	11.1	11.1
TN-1	48.7	52.0	24.0	88.1	88.1	64.8
LSD α= 0.05	5.76			20.71		

Table 8. Results of genotype evaluation for resistance to BLB pathotypes III, IV, and VIII in the generative phase based on SES from IRRI (2014).

Genotypes	P III		P IV		P VIII	
	Score	Criteria	Score	Criteria	Score	Criteria
HS4-11-1-73	1	R	3	MR	5	MS
HS1-35-1-4	1	R	1	R	5	MS
HS4-15-1-9	1	R	1	R	5	MS
HS4-15-1-16	1	R	1	R	5	MS
HS4-15-1-22	1	R	1	R	5	MS
HS4-15-1-24	1	R	3	MR	5	MS
HS4-15-1-26	1	R	1	R	5	MS
HS4-15-1-28	1	R	3	MR	5	MS
HS4-15-1-43	1	R	3	MR	5	MS
HS4-15-1-63	1	R	1	R	5	MS
HS4-15-1-70	1	R	1	R	5	MS
HS4-15-2-9	1	R	1	R	5	MS
Inpari 18	1	R	3	MR	3	MR
Inpari 34	1	R	3	MR	3	MR
Code	1	R	1	R	1	R
TN-1	7	S	9	HS	5	MS

Note: P= pathotype; R= resistant; MR= moderately resistant; MS= moderately susceptible; S= susceptible; HS= highly susceptible.

The resistance of genotypes tested against BLB in the generative phase ranged from resistant to highly susceptible, with a score of one to nine. Generally, the genotypes tested showed the best resistance to pathotype III, whereas the lowest resistance was TN-1 to pathotype IV, with a score of nine. Against pathotype III, all genotypes were resistant except TN-1 as susceptible (Table 8).

Code and eight DH lines attained resistant categories, while the other four lines (HS4-11-1-73, HS4-15-1-24, HS4-15-1-28, and HS4-15-1-43) have the same category as the commercial varieties being moderately resistant. The score of all DH lines resistance to BLB pathotype VIII was moderately susceptible, with a score of five, including TN-1. For TN-1, this number was lower than its resistance to pathotype III (score 5) and pathotype IV (score 9) in the generative phase.

DISCUSSION

Disease severity and intensity contributed to the resistance of a genotype to various BLB pathotypes. Disease severity used the numeric interval ranges of SES from IRRI (2014), reflecting genotype performances about BLB resistance based on the percent area affected by BLB symptoms. Meanwhile, disease intensity summarized the total effect of each *Xoo* pathotype on the genotypes tested. It explained why categorizing genotypes are different by SES from IRRI (2014) and somehow differed nonsignificantly in the LSD test. However, the LSD test calculated the most negligible significance between means, but SES from IRRI (2014) scale comprised the number of intervals.

Kadir (2009) stated that resistance to BLB varied depending on its virulence, determined by three components: the pathogen pathotype, the host (genotype), and the biotic and abiotic environments. It supports the study's results, with varied severity and intensity of the disease, influenced significantly the most by pathotype, followed by genotype, and the interaction between genotype and pathotype. It is also in line with Suryadi and Kadir (2009), who stated that a wide range of genetic variability in *Xoo* pathogens and their virulence explained different resistance responses of rice plants in particular geographic areas.

Generally, a DH line classifies more susceptible to pathotype VIII than pathotype

III and IV in both growth phases. Differences in resistance reactions between genotypes may refer to differences in the resistance genes contained in each genotype. According to Ou (1985), the resistance of rice genotypes to BLB has one or more dominant or recessive genes controlling it that it inherited. *Xa* resistance genes are effective genes previously used for BLB control in Asia since 1970, including *Xa3*/*Xa26*, *Xa4*, *xa5*, *Xa7*, and *Xa21* (Tasliah, 2012; Hu *et al.*, 2017). Plants with several resistance genes will respond well and not allow pathogens to develop. Screening against pathotype III yielded three DH lines (HS4-11-1-73, HS1-35-1-4, and HS4-15-1-22) resistant in the vegetative phase, with all DH lines resistant in the generative phase. Pathotype III *Xoo* was the dominant pathotype in South Sulawesi, Kalimantan, Java, and Bali. An earlier study reported having six virulent genes capable of breaking resistance genes (*Xa1*, *Xa2*, *Xa4*, *Xa10*, *Xa11*, and *Xa14*) in rice plants (Hifni and Kardin, 1998).

Pathotype IV *Xoo* was known to be highly virulent and became dominant throughout Java and Bali with the planting of the IR 64 variety in 1994, having virulence genes that are capable of breaking resistance genes in *Xa1*, *Xa2*, *Xa4*, *Xa7*, *Xa10*, *Xa11*, and *Xa14* rice plants. In this study, eight DH lines showed resistance to *Xoo* pathotype IV in the generative phase, namely HS1-35-1-4, HS4-15-1-9, HS4-15-1-16, HS4-15-1-22, HS4-15-1-26, HS4-15-1-63, HS4-15-1-70, and HS4-15-2-9, but none of the DH lines occurred resistant to *Xoo* pathotype IV in the vegetative phase. Given that pathotype IV is very virulent at the time of development, the level of BLB attack can vary according to time, location, cultivation technique, and the presence of inoculums at different stages of plant growth. In line with this study, TN-1, as a BLB-susceptible check, has the highest disease severity and intensity to pathotype IV in the generative phase. Screening for resistance genotypes to *Xoo* pathotype VIII revealed nonresistant DH lines. The pathotype VIII has virulence genes capable of breaking the resistance genes *Xa1*, *Xa2*, *Xa3*, *Xa4*, *Xa7*, *Xa10*, *Xa11*, and *Xa14*. Pathotype VIII appeared as the dominant pathotype in West Java. Thus, incorporating multiple genes into a single genotype, known as gene pyramiding, can guarantee broad-spectrum resistance to BLB of rice and the durability of the disease resistances since pathogens usually break single gene resistance after some time due to mutations or emergence of new races of pathogens (Jeung *et al.*, 2006; Chukwu *et al*., 2019).

Disease severity and intensity values in the generative phase were lower than in the vegetative (Tables 5 and 7). The differences could be due to the influence of inoculation time on BLB development. Khaeruni *et al.* (2014) reported differences in the severity of BLB in several rice varieties tested in two different growth phases due to the interaction between the severity and the inoculation time. BLB infection in the vegetative phase (5-8 weeks after planting) resulted in faster BLB development, especially in susceptible varieties. The average of BLB development reached 68.5% to 90% in the vegetative phase, while in the generative phase, the rate of disease development begins to slow down or stop. The slow BLB development rate can be due to the resistance plant structure, believed to have fully formed in the generative phase. Cao *et al.* (2020) stated that the lignin layer and the thickening of the cuticle on the epidermal cells increased the plant's resistance to *Xoo* and *Xoc* penetration. The lignification process was essential in the plant resistance mechanism to biotic and abiotic stress and the invasion of particular objects into plant structures (Sattler and Funnell-Harris, 2013). TN-1 (susceptible to *Xanthomonas*) had a thick leaf blade structure (78.33 µm), more metaxylem (63.60%), and fewer sclerenchyma layers (40%) and showed to be significantly different in form from resistant rice varieties, such as, IR36, Dular, and IR26 (Wahab *et al.*, 2022).

The lower disease severity and intensity in the generative phase may also be due to the accumulation of specific compounds related to defense mechanisms and their concentrations correlated with plant age. Phenolic acids, steroids, flavonoids, alkaloids, and terpenoids are secondary metabolites earlier reported acting as antimicrobials and

insecticides and have allelopathic functions (Goufo and Trindade, 2014). Phenolic acids, such as, phytoalexins, proved to be synthesized rapidly in high amounts in rice plants shortly after infection of the blast pathogen (*Pyricularia oryzae*) and reports of their concentrations positively correlated with rice blast resistance, whereas after *Xoo* infection, there was an increase in tannin and phenol content (Hasegawa *et al.*, 2010; Suharti and Leana, 2021).

The high severity of the BLB attack indicates a genotype susceptible to BLB. *Xoo* develops rapidly in susceptible genotypes, particularly humid conditions, causing blight symptoms (Yuriyah *et al.*, 2013). Du *et al.* (2022) reported a positive correlation between yield and leaf lesions in CNDH (Cheongcheong/Nagdong doubled haploid) rice lines caused by BLB. The greater the lesion on the leaf, the lower the yield and weight of the 1000 grains obtained. The yield and weight of 1000 grains appeared to be positively correlated, while the lesion length of the leaf had a direct effect on yield reduction.

Screening in two different growth phases found that eight out of 12 DH lines tested, i.e., HS4-11-1-73, HS1-35-1-4, HS4-15-1-9, HS4-15-1-16, HS4-15-1-22, HS4-15-1-24, HS4-15-1-26, and HS4-15-1-28, had resistance against pathotypes III and IV ranging from resistant to moderately resistant (Tables 5 and 8). However, all the DH lines tested revealed susceptibility and highly susceptible to pathotype VIII in the vegetative phase and moderately susceptible to pathotype VIII in the generative phase. Thus, two of the eight DH lines above were highly susceptible to pathotype VIII during the vegetative period (HS4-11-1-73 and HS4-15-1-22). Therefore, six DH rice lines, i.e., HS1-35-1-4, HS4-15-1-9, HS4-15-1-16, HS4-15-1-24, HS4-15-1-26, and HS4-15-1-28, can gain selection based on the scale of disease severity because they had resistance to pathotype III and IV.

Categories of Inpari 18 and Inpari 34 resulted as moderately resistant in the vegetative phase and resistant in the generative phase to *Xoo* pathotype III. In *Xoo* pathotype IV, Inpari 18 and Inpari 34 were comparatively resistant in both growth phases. Notably, Inpari 18 also categorizes as moderately susceptible, and Inpari 34 as susceptible in the vegetative phase against pathotype VIII, but in the generative phase, both classified as moderately susceptible. It was in line with Balitbangtan's (2019) varieties description: Inpari 18 was resistant to pathotype III, moderately resistant to pathotype IV, and susceptible to pathotype VIII; Inpari 34 was moderately resistant to pathotype III, susceptible to pathotype IV, and moderately susceptible to pathotype VIII.

Code (BLB resistance check) showed the best resistance response to BLB pathotypes III, IV, and VIII, which were resistant in both phases (Tables 5 and 8). Code (*Xa4* + *Xa7*) and Angke (*Xa4* + *xa5*) are two resistant BLB varieties already established and previously released in Indonesia. The results of this study consistently showed that the *Xa4* and *Xa7* resistance genes contained in Code are effective in suppressing BLB attacks. Davierwala *et al.* (2001) stated that the single major gene *Xa4* was one of the BLB-resistant gene sources for commercial resistance to BLB rice varieties, such as IR20 and IR64, for a long time before their resistance was broken. Deng *et al.* (2018) reported the distribution of *Xa4* and *Xa3*/*Xa26* in commercial hybrid rice varieties of India and China as sources of *Xoo* resistance. *Xa4* prevents the entry of *Xoo* into the plant through a cell wall-strengthened mechanism by encoding wall-associated kinases (WAKs) receptors (Hu *et al.*, 2017). The recently cloned by Chen *et al.* (2021) single major gene *Xa7* has shown to maintain resistance against BLB for a long time, be better compromised to high temperatures than other *Xa* genes, and has the potential to serve as a resistance gene in developing new resistant to BLB varieties (Webb *et al.*, 2010).

In this study, TN-1 validates as the susceptible genotype (moderately susceptible to highly susceptible) to BLB in both growth phases, with the highest disease severity and intensity, 70.0% and 92.2%, respectively. The result agrees with Suryadi *et al.* (2016), who revealed that TN-1 with the *Xa14* gene classifies as susceptible and has the most severe disease incidence (72.91%) in 15 *Xoo* isolates tested in Indonesia. Thus, in addition

to the influence of the host, pathotype, environment, and absence of resistance genes, the plant's vulnerable growth phase can influence high disease intensity.

CONCLUSIONS

The pathotype, followed by genotype, and their interactions substantially impacted the severity and intensity of BLB. Compared with the generative phase, occurring to be lower (1.7%–52% disease severity and 6.7%–88.1% disease intensity), they revealed higher in the vegetative phase (4.0%–70% disease severity and 13.0%–92.2% intensity). Six out of 12 DH lines, HS1-35-1-4, HS4-15-1-9, HS4-15-1-16, HS4-15-1-24, HS4-15-1-26, and HS4-15-1-28, could be beneficial selections based on their level of *Xoo* resistance, ranging from moderately resistant to resistant to pathotype III and IV at both growth phases.

ACKNOWLEDGMENTS

The authors thank the National Research and Innovation Agency (BRIN) and Indonesia Endowment Funds for Education (LPDP) for funding the research through Riset dan Inovasi untuk Indonesia Maju (RIIM) Scheme, contract number 18/IV/KS/06/2022 and 4830/IT3.L1/PT.01.03/P/B/2022, and the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor, for providing the greenhouse facility during this study.

REFERENCES

Acharya B, Sujata KC (2021). Field screening of rice (*Oryza sativa* L.) genotypes for resistance to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) disease. *Agric. Nat. Resour.* 4(1): 42-49. https://doi.org/10.3126/janr.v4i1.33201.

Anshori MF, Purwoko BS, Dewi IS, Ardie SW, Suwarno WB (2019). Selection index based on multivariate analysis for selecting doubled-haploid rice lines in lowland saline prone area. *SABRAO J. Breed. Genet*. 51(2): 161-174.

Anshori MF (2018). Characterization and selection of adaptive doubled haploid rice (*Oryza sativa* L.) lines to salinity stress. [Magister theses, IPB University]. Scientific Repository of IPB University.

BALITBANGTAN (Badan Penelitian dan Pengembangan Pertanian) (2019). Deskripsi varietas unggul baru padi. Balai Besar Padi Kementerian Pertanian Republik Indonesia.

BBPOPT (Balai Besar Peramalan Organisme Pengganggu Tumbuhan) (2021). Realisasi dan prakiraan serangan OPT utama padi, jagung dan kedelai di Indonesia MT 2020/2021. Direktorat Jenderal Tanaman Pangan, Kementerian Pertanian Republik Indonesia.

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): 187-198. https://doi.org/10.3390/pathogens9030187.

Chen X, Liu P, Mei L, He X, Chen L, Liu H, Shen S, Ji X, Zheng X, Zhang Y, Gao Z, Zeng D, Qian Q, Ma B 2021. *Xa7*, a new executor R gene that confers durable and broad-spectrum resistance to bacterial blight disease in rice. *Plant Communications* 2: 100143. https://doi.org/10.1016/j.xplc.2021.100143.

Chukwu SC, Rafii MY, Ramlee SI, Ismail SI, Oladosu Y, Okporie E, Onyishi G, Utobo E, Ekwu L, Senesie Swaray S, Jalloh M (2019). Marker-assisted selection and gene pyramiding for resistance to bacterial leaf blight disease of rice (*Oryza sativa* L.). *Biotech. & Biotech. Equip.* https://doi.org/10.1080/13102818.2019.1584054.

Davierwala AP, Reddy APK, Lagu MD, Ranjekar PK, Gupta VS (2001). Marker assisted selection of bacterial leaf blight resistance genes in rice. *Biochem Genet.* 39(7): 261-278. https://doi.org/10.1023/A:1010282732444.

Deng Y, Liu H, Zhou Y, Zhang Q, Li X, Wang S (2018). Exploring the mechanism and efficient use of a durable gene-mediated resistance to bacterial blight disease in rice. *Mol Breeding.* 38(18): 1-15. https://doi.org/10.1007/s11032-018-0778-1.

Ditlin (Direktorat Perlindungan Tanaman Pangan) (2011). Laporan tahunan 2010 Direktorat Perlindungan Tanaman Pangan. Dirjen Tanaman Pangan, Kementerian Pertanian Republik Indonesia.

Du XX, Park JR, Wang XH, Jan R, Lee GS, Kim KM (2022). Genotype and phenotype interaction between *OsWKRYq6* and BLB after

Xanthomonas oryzae pv. *oryzae* inoculation in the field. *Plants* 11(3): 287-302. https://doi.org/10.3390/plants11030287.

Fatimah, Prasetiyono J, Dadang A, Tasliah, Masumah (2019). Field evaluation of Conde progenies with early heading date and resistance to bacterial leaf blight. *Annales Bogorienses* 23 (2): 87-96. http://dx.doi.org/10.14203/ ann.bogor.2019.v22.n2.87-96.

Goufo P, Trindade H (2014). Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Sci Nutr*. 2(2)*:* 75-104. https://doi.org/10.1002/fsn3.86.

Hasegawa M, Mitsuhara I, Seo S, Imai T, Koga J, Okada K, Yamane H, Ohashi Y (2010). Phytoalexin accumulation in the interaction between rice and the blast fungus. *Molecular Plant-Microbe Interaction* 23(8): 1000-1011*.* https://doi.org/1000-1011.:10.1094/ MPMI -23-8-1000.

Herlina L, Silitonga TS (2011). Seleksi lapang ketahanan beberapa varietas padi terhadap infeksi hawar daun bakteri Strain IV dan VIII. *Bul. Plasma Nutfah* 17(2): 80-87. https://doi.org/10.21082/blpn.v17n2.2011. p80-87.

Hifni HR, Kardin MK (1998). Grouping of *Xanthomonas oryzae* pv. *oryza*e isolates using IRRI Near Isogenic Lines of rice. *HAYATI J Biosci*. 5: 66-72.

Hu K, Cao J, Zhang J, Xia F, Ke Y, Zhang H, Xie W, Liu H, Cui Y, Cao Y, Sun X, Xiao J, Li X, Zhang Q, Wang S (2017). Improvement of multiple agronomic traits by a disease resistance gene via cell wall reinforcement. *Nature Plants* 3: 1-9. https://doi.org/10.1038/nplants.2017.9.

IRRI (2014). Standard Evaluation System for Rice*.* International Rice Research Institute. 57 p.

Jeung JU, Heu SG, Shin MS, Cruz CMV, Jena KK (2006). Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathol*. 96(8):867-875. https://doi.org/ 10.1094/PHYTO-96-0867.

Ji Z, Wang C, Zhao K (2018). Rice routes of countering *Xanthomonas oryzae*. *Int. J. Mol. Sci.* 19: 1-14. https://doi.org/10.3390/ ijms19103008.

Kadir TS (2009). Controlling bacterial leaf blight by variety rotation. *Warta Penelitian dan Pengembangan Pertanian* 31: 1-3.

Kauffman HE, Reddy APK, Hsieh SPY, Merca, SD (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae* pv. *oryzae*. *Plant Diseases Rep* 57: 537-541.

Khaeruni A, Najamuddin E, Wijayanto T, Syair (2016). Resistance of local rice cultivars to bacterial leaf blight disease. *Jurnal Fitopatologi Indonesia.* 12: 89-93. https://doi.org/10.14692/jfi.12.3.89.

Khaeruni A, Taufik N, Wijayanto T, Johan EA (2014). Development of bacterial leaf blight disease inoculated on three varieties of paddy rice at various growth stage. *Jurnal Fitopatologi Indonesia*. 10: 119-125. https://doi.org/10.14692/jfi.10.4.119.

Lee SW, Han M, Park CJ, Seo YS, Jeon JS (2011). The molecular mechanisms of rice resistance to the bacterial blight pathogen, *Xanthomonas oryzae* pathovar *oryzae*. In J.C. Kader and D. Michel (Eds.), *Advances in botanical research* 60*:* 51-87. Academic Press.

Liu W, Liu J, Triplett L, Leach JE, Wang GL (2014). Novel insights into rice innate immunity against bacterial and fungal pathogens. *Annu. Rev. Phytopathol.* 52: 213-241. https://doi.org/10.1146/annurev-phyto-102313-045926.

Naqvi SAH (2019). Bacterial leaf blight of rice: An overview of epidemiology and management with special reference to Indian sub-continent. *Pakistan J Agri. Res.* 32(2): 359-380. 10.17582/journal.pjar/2019/32.2.359.380.

Ou SH (1985). Rice Disease. 2nd Edition. Commonwealth Mycological Institute.

Safitri H, Purwoko BS, Dewi IS, Ardie SW (2016). Anther culture to obtain rice lines tolerant to salinity. *J Agron Indo*. 44(3)*:* 221-227.

Sattler S, Funnell-Harris D (2013). Modifying lignin to improve bioenergy feedstocks: Strengthening the barrier against pathogens?. *Front. Plant Sci*. 4: 1-8. http://doi.org/10.3389/fpls.2013.00070.

Sudir, Yuliani D (2016). Composition and distribution of *Xanthomonas oryzae* pv*. oryzae* pathotypes, the pathogen of rice bacterial leaf blight in Indonesia. *AGRIVITA Journal of Agricultural Science* 38(2): 175-185. http://doi.org/10.17503/agrivita.v38i2.588.

Suharti WS, Leana NWA (2021). Induce resistance of rice plants against bacterial leaf blight by using salicylic acid application. *IOP Conference Series: Earth and Environmental Science*. 746: 1-6. http://doi.org/10.1088/ 1755-1315/746/1/012003.

Suryadi Y, Kadir TS (2009). Study on infection of *Xanthomonas oryzae* pv. *oryzae* on several rice genotypes: Relationships between

nutrition and disease intensity. *Ilmu Pertanian* 15(1): 26-36.

Suryadi Y, Samudra M, Priyanto TP, Susilowati DN, Lestari P, Fatimah, Kadir TS (2016). Determination of pathotypes from Indonesian *Xanthomonas oryzae* pv. *oryzae* population causing bacterial leaf blight and their reaction on differential rice. *Makara J. Sci.* 20(3): 109-118. https://doi.org/10.7454/mss.v20i3.6241.

Tasliah (2012). Resistance gene on rice to bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. *J Penelit Pengembangan Pertan.* 31: 103-112. http://dx.doi.org/10.21082/jp3.v31n3.2012.p%25p.

Wahab WA, Talip N, Basir S, Akbar MA, Saad MFM, Bunawan H (2022). Disease development and discovery of anatomically resistant features towards bacterial leaf streak in rice. *Agriculture* 12(5): 629-642. https://doi.org/10.3390/agriculture12050629.

Wang S, Liu W, Lu D, Lu Z, Wang X, Xue J, He X (2020). Distribution of bacterial blight resistance genes in the main cultivars and application of Xa_{23} in rice breeding. *Front. Plant Sci. 11*: 1-10. https://doi.org/10.3389/fpls.2020.555228.

Webb KM, Ona I, Bai J, Garrett KA, Mew T, Vera-Cruz CM, Leach JE (2010). A benefit of high temperature: Increased effectiveness of a rice bacterial blight disease resistance gene. *New Phytol.* 185(2): 568-576. https://doi.org/10.1111/j.1469-8137.2009.03076.x.

Yasmin S, Hafeez FY, Mirza MS, Rasul M, Arshad HMI, Zubair M, Iqbal M (2017). Biocontrol of bacterial leaf blight of rice and profiling of secondary metabolites produced by rhizospheric *Pseudomonas aeruginosa* BRp3. *Front Microbio.* 8: 2-23. https://doi.org/10.3389/fmicb.2017.01895.

Yuliani D, Amirrullah J, Sudir (2017). Keragaan penyakit padi pada varietas unggul baru untuk agroekosistem rawa dan lahan kering. *AGRIC* 29(1): 21-30.

Yuriyah S, Utami DW, Hanarida I (2013). Resistance test of promising rice lines against bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) Race III, IV, and VIII. *Buletin Plasma Nutfah* 19: 53-60.