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Evaluation of doubled haploid rice lines for agronomic performance and resistance to bacterial leaf blight

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Abstract. *Dewi IS, Putri RK, Purwoko BS, Yuriyah S, Lubis I. 2024. Evaluation of doubled haploid rice lines for agronomic performance and resistance to bacterial leaf blight. Biodiversitas 25: 4275-4283.* The dynamic of climate change aggravates the adverse effect of biotic stress. The biotic stresses affecting rice fields include bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). This research evaluated the agronomic performance and resistance of doubled haploid (DH) rice lines against three dominant *Xoo* pathotypes in Indonesia (i.e., pathotype groups III, IV, and VIII). Nineteen rice genotypes consisting of 14 advanced DH lines, two commercial varieties (Ciherang and Inpari 18), and three BLB checks (IRBB-66 as resistant check, IR-64 and TN-1 as susceptible check) were assessed against three dominant *Xoo* pathotypes in Indonesia following the SES for rice from IRRI. Results indicated that 11 DH lines, except M-1, M-4, and M-14 had good agronomic traits, including productivity over 8 tons ha^{-1}, significantly higher than Inpari 18. The varying severity of BLB was influenced considerably by pathotype, genotype, and pathotype-genotype interaction. The disease severity from each pathotype was relatively the same across two inoculation times (vegetative and generative phase). In both growth phases, all DH rice lines, except for M-5 and M-13 were categorized as resistant against pathotypes III and IV. These findings have practical implications for developing disease-resistant rice varieties, enhancing the relevance and applicability of the research.

Keywords: Agronomic traits, bacterial leaf blight, double haploid rice, disease severity, *Xoo* pathotype

Abbreviations: BLB: bacterial leaf blight; DH: double haploid; PH: plant height; NP: number of panicles per hill; FA: flowering age; HA: harvesting age; PL: panicle length; NTG: total number of grains; NFG: number of filled grains; FGP: filled grain percentage; W1000: 1000-grain weight; PROD: productivity. Bacterial leaf blight resistance scoring (HR: highly resistant, R: resistant, MR: moderately resistant, MS: moderately susceptible, S: susceptible, HS: highly susceptible)

INTRODUCTION

Global climate change and extreme weather events significantly impact crop production, leading to food shock that threatens global sustainability (Cottrell et al. 2019). These changes exacerbate abiotic and biotic stresses, limiting the plant life cycle factors (Oshunsanya et al. 2019; Surówka et al. 2020; Teshome et al. 2020; Skendžić et al. 2021). Reports indicate that biotic stresses, including pathogens and pests, cause yield losses of 17.2% to 30% (Savary et al. 2019). The long-term impacts of climate change affect pathogen sensitivity to temperature and humidity, impacting their growth, reproduction, spread, and severity of damage (Chattopadhyay et al. 2019). In Indonesia, rice is crucial for food security, necessitating the development of new high-yielding rice varieties resistant to biotic stress, offering hope for a more secure and sustainable food future.

According to the BBPOPT (2023), biotic stresses affecting rice fields include bacterial leaf blight (BLB), blast, brown planthoppers, rice stem borers, mice, and the tungro virus. BLB is the most destructive, caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). This pathogen has been reported to have an increased ability to infect hosts due to ideal temperatures for colonizing rice xylem, which are triggered by global temperature changes (Hunjan and Lore 2020). Nandal et al. (2005) noted that these changes lead to increased mutations in *Xoo* pathotypes, resulting in elevated levels of Extracellular Polysaccharides (EPS). EPS aids pathogens in self-propagation, protection against unfavorable environments, signaling, and energy storage, thus contributing to the rising threat of BLB. As a result of their rapid spread and environmental changes, BLB has significantly impacted rice production. Since the first report in Japan, BLB has spread to rice-producing countries across Southeast Asia, and several countries in Asia, Africa, America, and northern Australia (Naqvi 2019). Damage caused by BLB may reach up to 50% yield losses depending on the variety, growth phase, geographic location, and environmental conditions that favor infection (Fiyaz et al. 2022). BLB affected 22,630 ha of rice crops in Indonesia during 2023, representing 21.3% of the total area affected by the pest (BBPOPT 2023).

Various strategies for managing BLB using cultural and biological practices and the addition of technology are often incompatible with the crop production system or the regulatory environment (Sharma et al. 2022). Meanwhile, the large application of heavy, non-selective pesticides contributes to environmental degradation and potential resistance (Curutiu et al. 2017). Consequently, cultivating BLB-resistant plants is considered the most effective and cost-efficient method for controlling BLB. Currently, resistance to BLB is required to release new rice varieties in Indonesia.

Several studies have shown variations in the virulence of *Xoo* populations interacting with rice. These populations are classified into several pathotypes based on their ability to infect specific hosts. The ability of a rice genotype to overcome specific *Xoo* virulence factors is determined by the differences in the *Xa* genes it carries. Therefore, the *Xoo* pathotype is determined by the differences in the *Xa* genes carried by the rice genotype (Rashid et al. 2021; Yang et al. 2022). As of 1998, eleven *Xoo* pathotypes had been identified in Indonesia, with pathotype groups III, IV, and VIII being the most dominant (Sudir and Yuliani 2016). Given the diverse virulence among these pathotypes, previous studies have shown varying resistance levels among rice genotypes against BLB (Putri et al. 2023). This underscores the necessity of screening and quantifying resistance to assess candidate varieties' resistance to BLB. This research aimed to evaluate the agronomic performance and resistance of several promising doubled haploid (DH) rice lines from previous research against the three pathotype groups (III, IV, and VIII) of *Xoo*.

MATERIALS AND METHODS

Research materials

The yield trial was conducted in Beru Village, Wlingi District, Blitar, East Java, Indonesia, and BLB resistance screening in the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor greenhouse. A total of 19 rice genotypes were used in the study, consisting of 14 advanced DH rice lines (M-1 to M-14), 2 commercial varieties (M-15: Ciherang and M-16: Inpari 18), and 3 BLB as checks. The DH lines obtained from anther culture of several F_1s, i.e., F_1: Inpago 8×IR8770514-11-B-SKI-12 (M-1 to M-3); F_1: Inpago 8×IR83140-B-11-B (M-4 to M-9); F_1: B1111430D-MR-1-1-PN-3-MR-2-Si-3-PN×IR83140-B-11-B (M-10 to M-14). BLB resistance evaluation used 3 BLB check varieties (IRBB-66 as BLB resistant check, IR-64 and TN-1 as BLB susceptible check) and three dominant BLB pathotypes in Indonesia (Pathotypes III, IV, and VIII). BLB pathotype groups III, IV, and VIII isolates were obtained from the ICABIOGRAD collection.

Procedures

Yield trial of tested genotype

The yield trial was conducted using a randomized complete block design in three replications, with genotype as a factor. The experimental unit was a 4×5 m plot with a population density of 320 hills per plot. Maintenance includes replanting, irrigation arrangements, fertilization, and pest and disease management. Harvesting was conducted when 90% of the panicles in a single plot turned yellow. Observation was performed on five agronomic traits, including plant height (PH), number of panicles per hill (NP), days to flowering (FA), days to harvest (HA), and panicle length (PL). Additionally, five yield components were observed including the number of filled grains (NFG), the total number of grains (NTG), filled grain percentage (FGP), the weight of 1000 grains (W1000), and productivity (Prod). Ten sample plants were observed for each experimental unit, and one panicle per sample plant was observed.

Evaluation of BLB resistance

The evaluation was arranged in a randomized block design with two factors (genotype and pathotype). The entire experiment was replicated thrice so that there were 171 experimental units in total. Each rice genotype was germinated in a petri dish and sown in a nursery. After 14 days, the seedlings were transplanted into a pot with a diameter of 16 cm and a height of 19 cm, filled with paddy soil. Five seedlings were planted in a pot for each experimental unit to make 57 experimental units for each pathotype. The inoculum sources were grown on Wakimoto agar media, which contained per liter 0.5 g $Ca(NO_3)_2$, 2 g $Na_2HPO_4.12\,H_2O$, 5 g peptone, 15 g sucrose, 0.5 g $FeSO_4.7\,H_2O$, 15 g agar, and 1 L distilled water. The medium was incubated for 2×24 hours at room temperature. One colony of *Xoo* was used to obtain the inoculum source, which was then purified on the same medium. The *Xoo* was diluted with distilled water, and the cell suspension was adjusted to a concentration of 10^8 CFU mL^{-1}. The *Xoo* inoculation was performed using the clipping technique during the vegetative phase at 35 days after sowing (DAS) and the generative phase at 55-60 DAS. Disease severity was observed in each growth phase after two weeks of inoculation, following the IRRI SES for rice (IRRI 2013), and recorded in Table 1. Disease severity is calculated as the ratio of lesion length (cm) to leaf length (cm).

Data analysis

The data from the yield trial was analyzed using analysis of variance (ANOVA) and the least significant difference (LSD) test at a 5% significance level using the Statistical Analysis System on Demand for Academics (SAS-ODA). Then, a heatmap of Pearson correlation analysis was performed in the R-Studio package 'corrplot' that illustrated the correlation between agronomic traits. This helps identify important traits during selection. The BLB severity data were analyzed using ANOVA followed by the LSD test at a significance level of 5%. The disease resistance scores for BLB were then evaluated descriptively by comparing them with the scores of the BLB check.

Table 1. Resistance scoring based on disease severity due to BLB

Score	Severity (%)	Criteria	Category
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

RESULTS AND DISCUSSION

Yield and agronomic performance of tested genotypes

The yield and agronomic performance of the tested genotypes varied, except for the number of panicles per hill (Table 2). In general, the observed character values indicate good accuracy, as shown by the small coefficient of variation (CV) (1.5% - 7.1% < 20%). A low CV indicates environmental homogeneity and relatively high levels of observation accuracy.

The results showed that nine DH lines were within the ideal ideotype range for plant height, and they were not too tall (above 125 cm) or too short (below 80 cm). Plants shorter than 80 cm can be more challenging to care for and harvest. Farmers prefer plant heights between 80 and 125 cm (Kartina et al. 2019). Plants taller than 125 cm will be difficult to adopt because they easily lodge. Five DH lines M-1, M-2, M-8, M-9, and M-14 were found to have plant heights ranging from 129.4-149.6 cm, and therefore, they were not recommended.

M5 line showed an equivalent number of days to flowering and harvest as Inpari 18, which has the shortest flowering and harvest age, according to the LSD test. Ciherang was observed to have the most extended days to flowering and harvest compared to the other genotypes. However, the days to harvest in all genotypes ranged from 108 to 122 DAS, categorized as early maturing. The average panicle length of the test genotypes was 25.6 cm. Compared to the two commercial varieties, the genotypes M-7, M-8, M-9, M-10, M-13, and M-14 have significant panicle lengths. Their panicle lengths ranged from 26.5 to 27.4 cm. The remaining genotypes had panicle lengths equivalent to the two commercial varieties.

The DH lines M-11, M-12, and M-13 had the highest filled grain. These lines, along with four other lines, namely M-7, M-8, M-10, and M-14, were found to have a significantly higher number of filled grains than the two commercial varieties. The M-13 DH line had the highest total grain number (336.2 grains per panicle); in addition, nine DH lines, including M-3, M-7, M-8, M-9, M-10, M-11, M-12, M-13, and M-14, had a significantly higher total grain number than the two commercial varieties. Although the M-13 DH line had a high total number of grains, it had an average percentage of filled grains of 73.4%. M-11 and M-12 had high-filled grains at 80.6% and 85.3%, respectively. The commercial varieties, namely, Ciherang and Inpari 18, had a percentage of filled grain ranging from 80.0% to 81.2%.

The Inpari 18 variety had fewer grains and a lower filled grain percentage than the other genotypes, but it had the highest 1000-grain weight of 32.5 g. Ciherang had a 1000-grain weight in the range of 26.2 g. The M-1, M-2, M-4, M-11, M-12, and M-14 DH lines had a 1000-grain weight significantly higher than Ciherang, while the M-3, M-5, M-6, M-7, M-8, and M-13 DH lines had a 1000-grain weight equivalent to Ciherang.

Table 2. Average of agronomic characters and yield of tested genotype

Genotype	PH	NP	FA	HA	PL	NTG	NFG	FGP	W1000	Prod
M1	146.0	19.9	93.3	121.7	25.2	163.6	125.8	76.8	28.7	6.4
M2	134.2	21.4	92.7	120.7	26.0	173.9	138.5	80.0	27.9	9.3
M3	112.1	21.8	90.7	119.0	25.0	237.3	165.2	69.9	26.3	9.4
M4	113.2	17.8	85.3	110.7	25.9	224.5	161.6	72.0	28.5	6.7
M5	88.5	19.7	85.0	108.3	24.4	183.3	156.4	85.4	26.9	8.2
M6	88.9	21.0	86.0	109.3	24.1	193.5	166.5	86.1	25.5	8.2
M7	121.5	22.1	90.7	115.7	26.5	229.6	191.0	83.1	26.9	9.5
M8	149.6	19.5	89.7	118.7	27.4	236.5	184.7	78.1	26.8	8.6
M9	143.7	20.1	89.7	114.3	27.2	245.8	174.3	70.9	25.3	8.2
M10	114.9	21.4	93.3	120.0	26.8	245.5	192.7	78.6	25.3	8.7
M11	114.5	20.7	92.7	117.0	24.4	282.4	227.8	80.6	27.8	9.5
M12	112.6	19.0	92.7	117.0	24.0	270.6	230.8	85.3	28.4	9.4
M13	123.4	21.3	93.3	118.0	26.8	336.2	246.7	73.4	26.7	10.0
M14	129.4	20.8	94.0	120.0	26.5	267.7	192.6	72.0	27.3	7.9
Ciherang	114.8	21.9	95.0	122.0	25.1	204.0	165.6	81.2	26.2	9.0
Inpari 18	98.8	21.3	83.0	108.3	24.6	199.2	159.2	80.0	32.6	7.2
Average	119.1	20.6	90.4	116.3	25.6	230.8	179.9	78.3	27.3	8.5
Pr > F	< 0.001**	0.0604ns	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**
CV (%)	2.7	7.1	1.9	1.5	2.6	5.9	5.8	3.9	1.8	5.0
LSD	5.4	-	2.8	2.9	1.1	22.7	17.5	5.2	0.8	0.7

Notes: PH: plant height (cm); NP: the number of panicles per hill; FA: flowering age (day after sowing); HA: harvesting age (day after sowing); PL: panicle length (cm); NTG: the total number of grains; NFG: the number of filled grains; FGP: filled grain percentage; W1000: 1000-grain weight (g); Prod: productivity (t ha^{-1}); CV: coefficient of variation (%); ** highly significant different at α=0.01; ^{ns}: not significantly different at α=0.05

Productivity of all DH lines, except for the M-1, M-4, and M-14, were found to have a productivity of more than 8.0 tons ha^{-1}. Among the 11 lines, M-13 was significantly higher than Ciherang. Five lines (M-2, M-3, M-7, M11, and M-12) were equivalent to Ciherang. The productivity of those lines and 5 other lines (M-5, M-6, M-8, M-9, and M-10) were higher than Inpari 18. Correlation between yield and agronomic traits can help to identify traits during selection. For instance, a Pearson correlation analysis between all observed traits found that the total number of grains, filled grains, and panicles per hill were positively and significantly correlated with yield (Figure 1). This means that increasing the number of grains, the number of filled grains, and the number of panicles per hill can lead to higher yields. Similar research by Yadi et al. (2021) showed a strong and positive correlation between yield and the number of panicles per square meter. The results of the correlation analysis in this experiment also found a robust correlation (symbolized by the red color on the heatmap visualization in Figure 1) and a significant correlation in the days to flowering and the days to harvest, number of filled grains, and total number of grains per panicle. This helps explain that genotypes with a short flowering period, which refers to the time from the onset days of flowering to the end of flowering, such as Inpari 18, tend to have the shortest harvest period. At the same time, the high number of filled grains contributes positively to the total of grain produced or productivity.

Resistance of tested genotype against BLB

The analysis of variance indicates that genotype, pathotype, and their interaction significantly influenced disease severity in both the vegetative and generative phases (Table 3). Specifically, the significant values associated with each source of variance in disease severity. The results indicate that differences among *Xoo* pathotypes were the most substantial factor influencing disease severity, accounting for 59.7% to 79.1% of the observed variation in both growth phases. This finding underscores that pathotype differences are crucial in understanding variability in disease severity. Rashid et al. (2021) reported that each *Xoo* pathotype possesses distinct virulence levels. According to Ashwini et al. (2024), different pathotypes of *Xoo* have varying levels of virulence, which affects their aggressiveness, with some causing severe symptoms and significant yield losses, while others result in milder disease. The effectiveness of resistance observed in the study might not fully extend to areas where other pathotypes, such as I, II, V, VI, and VII, are more prevalent or where environmental factors significantly differ, potentially influencing the expression of resistance. However, extensive testing of *Xoo* isolates from across Indonesia has consistently identified pathotypes III, IV, and VIII as dominant in major rice-growing areas such as Java, Bali, and Sulawesi (Yuriyah et al. 2013; Yuriyah and Utami 2015). This consistent dominance suggests that these pathotypes represented the high pressures of BLB disease in Indonesia. Thus, while localized adaptation and testing are important, the study's focus on these dominant pathotypes supports the broader applicability of the resistance findings to Indonesian rice cultivation.

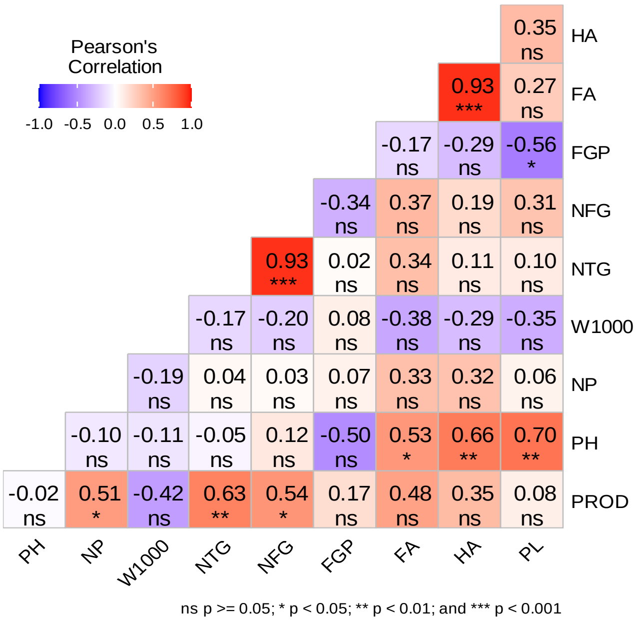

Figure 1. Heatmap correlation between yield and agronomic traits in the tested rice genotypes. Note: PH: plant height; NP: number of panicles per hill; W1000: 1000-grain weight; NTG: total number of grains; NFG: number of filled grains; FGP: filled grain percentage; FA: flowering age; HA: harvesting age; PL: panicle length; PROD: productivity

Table 3. Analysis of variance for BLB disease severity in vegetative and generative phases

Source of variance	Vegetative		Generative	
	Pr > F	Contribution (%)	Pr > F	Contribution (%)
Replication	.0003**	0.5	0.0011**	0.8
Genotype	<.0001**	10.4	<.0001**	13.4
Pathotype	<.0001**	74.4	<.0001**	59.7
Genotype×Pathotype	<.0001**	11.5	<.0001**	19.7

Notes: **: highly significant different at α=0.01; *: significantly different at α=0.05

The interaction between rice genotypes and *Xoo* pathotypes is crucial, as certain pathotypes can overcome specific resistance genes (Du et al. 2022). Furthermore, in the case of rice-growing areas, *Xoo* populations often consist of multiple pathotypes, and these can coexist within the same region, affecting disease severity based on the local pathogen composition (Rashid et al. 2021; Amin et al. 2023; Kanipriya et al. 2024). Over time, these pathotypes can evolve, causing resistance in previously resistant cultivars to break down (Diallo et al. 2023), thus emphasizing the importance of continuous monitoring and updating of resistance strategies.

Several genotypes were categorized from highly resistant (HR) to moderately resistant (MR) observed in vegetative and generative growth phases. The number of genotypes showing highly to moderately resistant (HR to MR) responses in the vegetative and generative phases has little contrast among the three *Xoo* pathotypes tested (Figure 2). Slight differences were observed between pathotype groups IV and VIII, but no specific pattern was detected, indicating differences in resistance between the two growth phases. These findings lead to the potential to significantly impact our understanding of resistance genotypes in different growth phases.

Plant age is a crucial factor in determining the outcome of a host-pathogen interaction. Several studies have shown that the level of resistance of rice plants to BLB disease can vary depending on the growth phases. According to Ansari et al. (2019), when the *Xoo* pathogen was inoculated, it was observed that disease severity and yield loss decreased as the rice plants progressed through different growth phases. The pathogen affected the number of tillers, time of panicle initiation, booting, flowering, and heading. Noor et al. (2006) also found that leaf inoculation treatment of BLB during the germination stage on basmati rice resulted in the highest disease severity at 89.5%. In contrast, injection at the leaf flag stage resulted in a disease severity of 56.21%. Leaf stage-associated resistance demonstrated that hundreds of genes are involved in defense responses, phytohormone biosynthesis and signaling, and calcium signaling (Xu et al. 2018; Zhong et al. 2024).

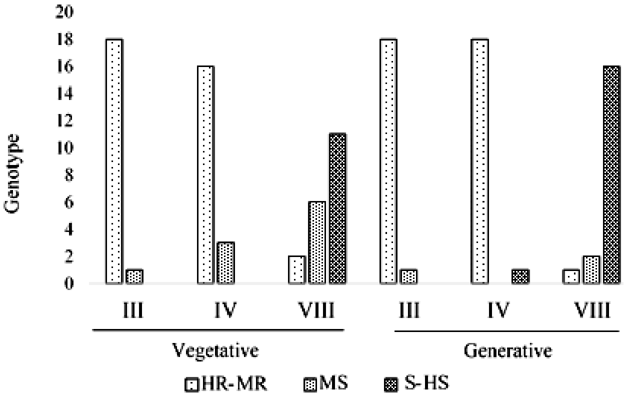

Figure 2. The number of genotypes and resistance categories in two growth phases. Notes: HR: highly resistant, R: resistant, MR: moderately resistant, MS: moderately susceptible, S: susceptible, HS: highly susceptible. Scoring follows IRRI (2013)

The improvement of multiple agronomic traits by a disease-resistance gene via cell wall reinforcement has been reported by Hu et al. (2017) and Shamsunnaher et al. (2020). Chronological age is calculated based on the number of times after planting known as Days After Planting (DAP) or Weeks After Planting (WAP). In contrast, physiological age is characterized by the appearance or presence of specific morphological and physiological characters at the onset of the experiment. In the model plant, *Arabidopsis* at physiological age showed that the juvenile phase of the leaves produced higher disease severity (Xu et al. 2018). Age-related resistance (ARR) is reported to be associated with organ maturation in plants related to strengthening the 'physical barrier' and resistance based on chemical compounds, and it is related to changes in the vegetative phase. Rice with ARR was reported to have better post-vegetative resistance through down-regulation of Gibberellin (GA) activity, which is associated with susceptibility (GA contradicts resistance based on jasmonic acid synthesis) (Yimer et al. 2018; Liu et al. 2019). In contrast, the excellent resistance obtained in this study at the beginning of the growth phase is of interest for further studies regarding the resistance mechanism. One of the resistance mechanisms of particular note is the regulation of the *Xa* gene (Xa_{10}, Xa_{23}, or Xa_{27}) related to hypersensitive response, which causes local death of infected cells, thereby inhibiting the development of pathogens (Zhang et al. 2015) and can achieve systemic immunity in plants (Joshi et al. 2020; Bakade et al. 2021; Xu et al. 2021).

The severity of disease caused by pathotype groups III and IV of *Xoo* in the vegetative and generative phases was relatively lower than pathotype VIII (Figures 3 and 4). During the generative phase, only the resistant check (IRBB-66) showed the lowest severity (5.6%) to pathotype VIII. IRBB-66 showed the best resistance to the three *Xoo* pathotypes tested at two growth phases (2.6-7.8%). IRBB-66 was reported to have several recessive and dominant resistance genes (R-gene), namely, Xa_{21}, xa_{13}, Xa_{7}, xa_{5}, and Xa_{4} (Suryadi and Kadir 2017). Higher and more durable resistance can be achieved by having multiple R genes in a single variety. Even if the resistance regulated by one gene was broken, other resistance genes could still overcome *Xoo* virulence (Kumar et al. 2020). Approximately 49 BLB resistance genes have been identified or mapped (Chen et al. 2020; Pradhan et al. 2020; Xing et al. 2021). The development of resistant varieties is currently directed at the genetic constitution of a single R-gene or pyramiding several R-genes in one variety (Pradhan et al. 2022; Ullah et al. 2022), as in IRBB-66.

The resistance of a host to pathogens can be triggered by certain factors and is regulated by genes such as *Xa*. This type of resistance is called 'inducible' as it requires a trigger to be activated. The "gene for gene hypothesis" explains how this works: a specific gene from the pathogen and a resistance gene from the host must complement or cognate to activate resistance. If complementation does not occur, the host will fail to activate the resistance system, and the plant will become susceptible to the pathogen (Flor 1971). Differences in the resistance level of various test genotypes to the same pathotype can be observed due to the absence of the R-gene, which regulates the receptors to recognize the susceptible gene elicitor. Additionally, the resistance level of a test genotype to different pathotypes can vary since the test genotype does not have a single R-gene that matches the susceptible gene elicitor.

Referring to the R-gene composition of IRBB-66, this genotype has a complete R-gene class ($Xa_{21=}$LRR-RLK; $xa_{13=}$SWEET-type protein; $Xa_{7=}$R protein executor; $Xa_{5=}$TFIIAγ5 transcription factor; $Xa_{4=}$Wall-associated kinase or WAK) (Chukwu et al. 2019; Jiang et al. 2020; Kumar et al. 2020; Alvarez-Martinez 2021; Chen et al. 2021). Based on the three major classes of R genes, Xa_{21} regulates resistance when it cognates with the Avr (avirulence) gene *RaxX*. Xa_{21} of the RLK class acts as a transmembrane receptor that captures the *Xoo*-secreted *RaxX* effector and subsequently activates resistance mechanisms. SWEET protein is a transporter protein hijacked by PthXo$_1$ (*Xoo* Avr gene) through the secretion of TALe (transcription activator-like effector). Next, TALe will target EBE (effector binding protein) to hijack the SWEET protein that pathogens use to colonize. The pairing of xa_{13} with PthXo$_1$ will block EBE (effector binding protein) from being recognized by TALe so that piracy cannot occur. The mechanism of immune activation by xa_{13} is quite similar to the executor R protein (Xa_7); TALe will pair with the EBE on the Xa_7 promoter and then activate the resistance system. The completeness of the R-gene class that accommodates different resistance mechanisms in IRBB-66 means this variety is widely used as a resistance check in BLB resistance evaluations, including in this research.

Fourteen DH rice lines tested against pathotype III of *Xoo* showed resistant to moderately resistant (R to MR) scores in two growth phases. All rice lines, except for M-5, and M-13, were resistant against pathotype groups III, and IV of *Xoo* in both vegetative and generative phases, because M-5 and M-13 were categorized as moderately susceptible (MS) to pathotype IV of *Xoo* during the generative phase (Table 4). M-10 and M-11 were superior to Ciherang, and IR-64.

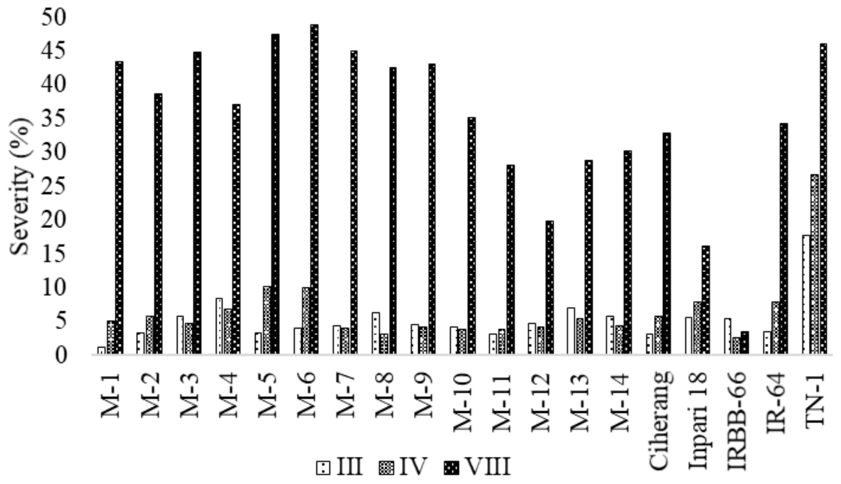

Figure 3. Disease severity response to pathotypes III, IV, and VIII of *Xoo* in vegetative phase; LSD at α=0.05: 5.79

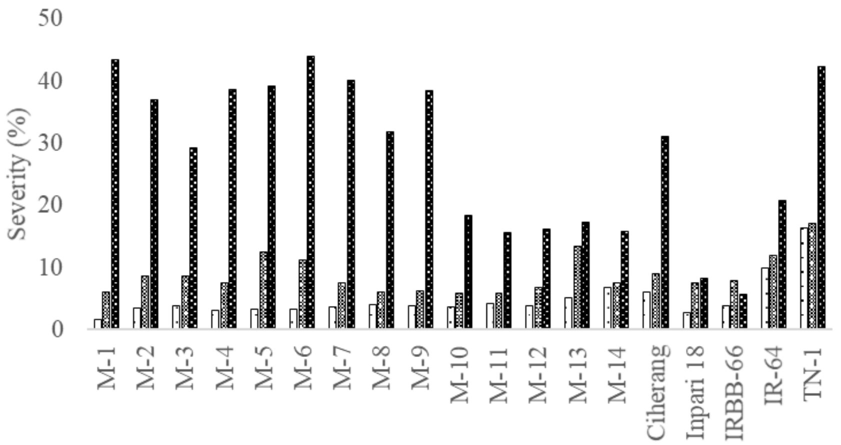

Figure 4. Disease severity response to pathotypes III, IV, and VIII of *Xoo* in generative phase; LSD at α=0.05: 6.58

Table 4. Resistance scoring of tested genotypes to pathotypes III, IV, and VIII of *Xoo* in the vegetative and generative phases

Genotype	Vegetative						Generative					
	III		IV		VIII		III		IV		VIII	
	Score	Category	Score	Category	Score	Category	Score	Category	Score	Category	Score	Category
M-1	1	R	1	R	7	S	1	R	3	MR	7	S
M-2	1	R	1	R	7	S	1	R	3	MR	7	S
M-3	1	R	1	R	7	S	1	R	3	MR	7	S
M-4	3	MR	3	MR	7	S	1	R	3	MR	7	S
M-5	1	R	3	MR	7	S	1	R	5	MS	7	S
M-6	1	R	3	MR	7	S	1	R	3	MR	7	S
M-7	1	R	1	R	7	S	1	R	3	MR	7	S
M-8	3	MR	1	R	7	S	1	R	1	R	7	S
M-9	1	R	1	R	7	S	1	R	3	MR	7	S
M-10	1	R	1	R	7	S	1	R	1	R	5	MS
M-11	1	R	1	R	7	S	1	R	1	R	5	MS
M-12	1	R	1	R	5	MS	1	R	3	MR	5	MS
M-13	3	MR	1	R	7	S	1	R	5	MS	5	MS
M-14	1	R	1	R	7	S	3	MR	3	MR	5	MS
Ciherang	1	R	1	R	7	S	1	R	3	MR	7	S
Inpari 18	1	R	3	MR	5	MS	1	R	3	MR	3	MR
IRBB-66*	1	R	1	R	1	R	1	R	3	MR	1	R
IR-64*	1	R	3	MR	7	S	3	MR	3	MR	5	MS
TN-1*	5	MS	7	S	7	S	5	MS	5	MS	7	S

Notes: R: resistant, MR: moderately resistant, MS: moderately susceptible, S: susceptible, BLB check (Resistant: IRBB-66, Susceptible: IR-64 and TN-1)

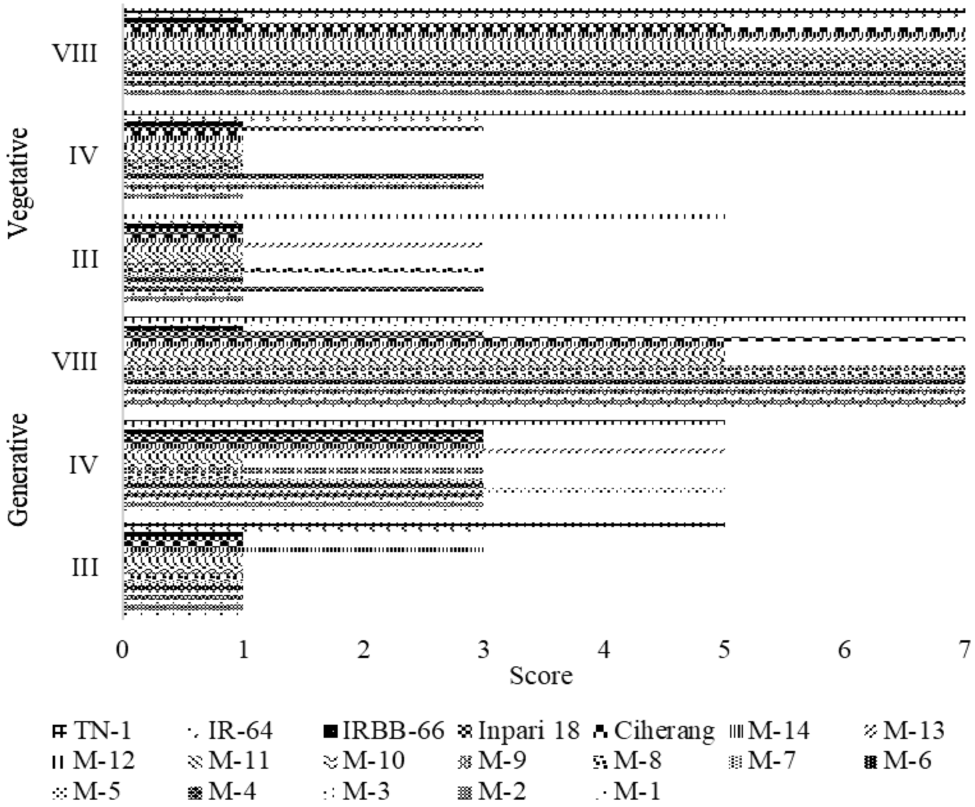

Figure 5. Tested genotype scoring for pathotypes III, IV, and VIII of *Xoo* in vegetative and generative phases

Similar to this study, other research, when evaluating the resistance of several new high-yielding rice varieties to BLB disease, produced diverse resistance responses to pathotype groups III, IV, and VIII of *Xoo* (Rumanti et al. 2016; Sutrisno et al. 2018). Considering that BLB pathotypes III, IV, and VIII are dominant in major rice-growing areas in Indonesia, Putri et al. (2023) recommended that several genotypes ranging from resistant to moderately resistant to pathotype groups III and IV of *Xoo* should be released as new rice varieties. This is also due to the three dominant *Xoo* pathotype groups found in Indonesia, pathotype IV is the most virulent against all differential varieties (Suryadi et al. 2016), possessing nine virulence genes. According to the 'gene for gene hypothesis', even if there is a complete class of *Xa* genes, there may be types of *Xa* and Avr genes that are not complementary, thus creating vulnerability (Joshi et al. 2020). In this study, the response of check variety IRBB-66 to the three BLB pathotypes is better compared with other genotypes (Table 4 and Figure 5). All DH lines, except M-4 to M-6 demonstrated better resistance than Inpari 18 against pathotype IV during the vegetative phase. In the generative phase, all DH lines and Inpari 18, except for M-5 and M-13 exhibited good resistance to pathotypes IV (Figure 5).

In conclusion, the productivity of 11 DH lines, except for the M-1, M-4, and M-14, was more than 8 tons ha^{-1}, significantly higher than Inpari 18, with good agronomic traits. The number of filled grains, the total number of grains, and the number of panicles per hill can be used as selection criteria. Differences in *Xoo* pathotypes contribute the most to differences in BLB disease severity. All DH rice lines, except for M5 and M13, have been found to possess resistance against pathotype groups III and IV of *Xoo* in both vegetative and generative phases. All DH rice lines with good agronomic traits, high productivity of more than 8 tons ha^{-1}, and resistance against pathotype groups III and IV of *Xoo* can be selected further as candidates for new rice varieties.

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