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INTROGRESSION OF THE *LYZ-C* GENE FROM THE TRANSGENIC *JAPONICA* RICE CULTIVAR 'KINLYS 1/3/23' INTO THE ELITE *INDICA* RICE CULTIVAR 'CIHERANG'

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SUMMARY

C-type lysozyme, which is encoded by the chicken egg *LYZ-C* gene, can degrade bacterial cell walls. The *LYZ*-C gene from the transgenic *japonica* rice 'KinLys 1/3/23' was successfully introgressed into the elite *indica* rice cultivar 'Ciherang' via crossing and backcrossing. Selection on Murashige Skoog medium containing 30 mg/L hygromycin showed that the *hpt* gene, which is linked to the *LYZ*-C gene, segregated at the ratio of 1:0 in the F1 generation and 1:1 in the BC1F1 generation. This result confirmed that the transgene was stably inherited in a Mendelian pattern with a dominant single-copy gene. PCR analysis proved that the *LYZ-C* gene had been integrated into the genomes of the F1, BC1F1, and BC1F2 plants. Resistance against *Xanthomonas oryzae pv. oryzae* (*Xoo*) pathotype IV, the causal agent of bacterial leaf blight (BLB), was investigated in the transgenic BC1F2 population. Results showed that backcrossed plants had higher resistance to *Xoo* pathotype IV than the recurrent parent, indicating that the *LYZ-C* gene and BLB resistance trait had been successfully introgressed from 'KinLys 1/3/23' into the BC1F2 generation.

Keywords: Bacterial leaf blight, backcrossing, gene expression

Key findings: Crossing between the transgenic *japonica* rice 'KinLys1/3/23' containing the *LYZ-C* gene and *indica* 'Ciherang' rice, followed by backcrossing to 'Ciherang' rice increased the resistance of the BC1F2 generation to BLB caused by Xoo pathotype IV.

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INTRODUCTION

Different diseases limit rice production. Bacterial leaf blight (BLB), which is caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*), is one of the most destructive bacterial diseases of rice. The widespread cultivation of high-yielding varieties of rice increases the incidence of *Xoo* infection. Yield losses due to this disease vary depending on rice variety, environmental condition, and rice growth stage at the time of infection (Noh *et al.*, 2007; Suryadi *et al.,* 2011; Khan *et al.*, 2015;

Vemanna *et al.,* 2019). The annual reduction in global rice production due to BLB is approximately 20%–30% (Nino-Liu *et al.,* 2006). Approximately 0.24% (20 825 Ha) of the total rice production area in Indonesia (8 681 191 Ha) was infected by *Xoo* in mid-2019 (Ditlintan, 2019).

The use of resistant cultivars is considered to be the most effective method of controlling BLB disease. 'Ciherang' is an elite *indica* rice cultivar originating from Indonesia. It is widely cultivated because of its agronomic characters, such as resistance to brown planthopper biotypes 2 and 3, early maturity, high productivity, and attractive rice flavor. 'Ciherang' is cultivated in 37% of the total irrigated rice area in Indonesia. It is moderately resistant to *Xoo* pathotype III in the dry season and susceptible in the wet season and highly susceptible to pathotype IV in the dry and wet seasons (Yuliani *et al*., 2014). However, after several years of release, the resistance of 'Ciherang' has been broken down by *Xoo*. Thus, improving the resistance of 'Ciherang' to *Xoo* pathogens is important.

New hybrid rice that is resistant to a broad spectrum of *Xoo* pathotypes and capable of surviving for long periods can be developed by expressing the genes that encode antimicrobial compounds in rice plants. Lysozyme, an ubiquitous enzyme encoded by the *LYZ-C* gene, can hydrolyze the β-1, 4 glycoside bond between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycans found in Gram-positive and Gram-negative bacterial cell walls (Ibrahim *et al.,* 2002). *Indica* rice is more difficult to subject to genetic transformation than *japonica* rice because of its low regeneration potential in tissue culture (Ge *et al.,* 2006; Rahmawati *et al.,* 2010; Ngoc and Mishra, 2015). Ochieng (2014) successfully introduced the *LYZ*-C gene into the *japonica* rice cultivar 'Kinmaze'. The expression of the *LYZ-C* gene in the T2 and T3 generations of the transgenic 'Kinmaze' variety resulted in an increase in resistance to BLB. Among all transgenic lines, 'KinLys 1/3/23' shows the highest level of *LYZ-C* expression (Mustamin, 2017). In a previous study, we successfully introgressed the *Pita-2* gene from *japonica* rice 'IRBLta2-Re' into the 'Ciherang' cultivar via conventional breeding to obtain 'Ciherang' rice with resistance to blast disease (Fitriah *et al*., 2019). The objective of this research was to introgress the *LYZ-C* gene from the transgenic *japonica* rice 'KinLys 1/3/23' into the *indica* rice cultivar 'Ciherang' to improve resistance to BLB.

MATERIALS AND METHODS

Plant material

The elite *indica* rice cultivar 'Ciherang' was used as the recurrent parent (RP). The transgenic *japonica* breeding line 'KinLys 1/3/23' containing the *LYZ-C* gene was used as the donor parent (DP).

Pathogen strains used to test rice resistance to BLB

Xoo pathotype IV, one of the dominant *Xoo* pathotypes found in rice fields in Indonesia, was used as a pathogen to test rice resistance to BLB. The strain was provided by the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Ministry of Agriculture, Republic of Indonesia.

Crossing and backcrossing

Crosses were made between 'Ciherang' as the female parent and 'KinLys 1/3/23' as the male parent, and the obtained F1 plants were backcrossed with 'Ciherang' as the female parent to obtain BC1F1. The BC1F1 plants were allowed to self-pollinate to obtain the BC1F2 generation. Five hygromycin-resistant progeny lines of BC1F2 carrying the *LYZ-C* gene were analyzed. All transgenic plants were cultivated in the screen house under normal conditions.

Analysis of segregation in F1 and BC1F1 generations

Segregation analysis was performed on the basis of the heredity pattern of the hygromycin phosphotransferase (*hpt*) selectable marker gene that is linked to the *LYZ-C* gene. Sterilized seeds (F1 or BC1F1) were grown on Murashige and Skoog (MS) medium containing 30 mg/L hygromycin. After 4 weeks, the resistant and sensitive rice plants were scored, and then the segregation ratio was compared with the expected ratio of 1:0 for F1 and 1:1 for BC1F1 for one hybrid cross by using the chi-square test (χ^2) with the following formula:

$$X^2 = \sum \frac{(O-E)^2}{E}$$

where O = observation value, and E = estimation value.

DNA extraction and PCR analysis

Genomic DNA was isolated from rice leaves by using the modified CTAB method (Doyle and Doyle, 1990). DNA concentration was quantified with NanoVue Plus™ Spectrophotometer (Biochrom, UK). The integration of the *LYZ*-C gene into the F1, BC1F1, and BC1F2 plants was analyzed by using specific primer pairs designed to detect the chicken lysozyme gene cassette, i.e., 35S-F (5'-AAACCTCCTCGGATTCCATT-3') and Lys359-R (5'-TTCACGCTCGCTGTTATGTC-3'). The 35S-F primer was designed on the basis of the 35S CaMV promoter found in GenBank: AJ007626.1 (https://www.ncbi.nlm.nih.gov/nuccore/AJ007626.1). The Lys359-R primer is located at nucleotides 371–390 from the start codon of the lysozyme cDNA (Jung *et al*., 1980). PCR was performed for 35 cycles under the following conditions: pre-PCR at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 52 °C for 45 s, extension at 72 °C for 1 min, and post-PCR at 72 °C for 5 min. An ultraviolet transmission device was used to observe the PCR product after separation via 1% agarose gel electrophoresis.

Identification of the resistance of BC1F2 to *Xoo* pathotype IV

The BC1F2 plants carrying the *LYZ-C* gene were grown in a screen house. *Xoo* pathotype IV was grown by transferring 50 µL of *Xoo* stock to peptone, sucrose, and agar (PSA) medium and incubated at 28 °C for 3 days until a thin biofilm was formed. Two days before inoculation, *Xoo* from the original PSA mediumwas transferred into fresh PSA medium and incubated at 28 °C. On the day of the inoculation, the *Xoo* bacterial suspension was prepared by dissolving bacterial colonies in 10 mM sterilized $MgCl_2$ buffer until the OD_{600} of the solution reached 0.5. Fully expanded leaves of 50-day-old BC1F2 rice plants were inoculated through the leaf clipping method (Kauffman *et al*., 1973). Disease reaction was scored on the basis of the Standard Evaluation System of Rice (IRRI, 2013). Disease severity was evaluated at 14 days after inoculation (DAI) in accordance with the following formula:

$$\text{Disease severity} = \frac{\text{total necrosis length}}{\text{total leaf length}} \times 100\%$$

RNA isolation and cDNA synthesis

Total RNA was isolated from the young leaves of BC1F2 plants and controls ('Ciherang' and 'KinLys 1/3/23'). RNA was extracted by using Trizol® reagent in accordance with the manufacturer's instructions (Invitrogen®, USA). cDNA was synthesized by using the Toyobo® cDNA synthesis kit in accordance with standard procedure. The rice actin gene was amplified as an internal control by using the primer pair cAct-F (5'-GGATGCCTATGTGGGTG-ATG-3') and cAct-R (5'-ATTTACACTCGCGCATGCTA-3'). These primers were located at exons 2 and 3 of the actin gene. The amplification

of cDNA by using this pair of primers provides a 377 bp product, whereas the amplification of genomic DNA by using the same pair of primers yields a 492 bp product. Therefore, this pair of primers can be used to verify the purity of cDNA.

Analysis of *LYZ-C* gene expression in backcross populations

The expression of the *LYZ-C* gene in transgenic plants was qualitatively and quantitatively analyzed through PCR and qRT-PCR, respectively. PCR and qRT-PCR were carried out by using the total cDNA as a template, the primer pairs Lys-F (5'-GCACTAGTGGCAACATGAGGTCTTTGC-3') and Lys150-R(5'-CACACCCAGTTTCCCAGGCT-3') under the same reaction conditions. The Lys-F primer is located 14 nucleotides before and 10 nucleotides after the start codon, and Lys150-R is located at nucleotides 123–143 from the start codon of the cDNA of lysozyme (Jung *et al*., 1980). The rice actin gene was used as the internal control. The qRT-PCR mixture comprised 1 µL of cDNA (50 ng), 5 µL of SsoFast™ EvaGreen® Supermix, 2.5 pmol forward primer, 2.5 pmol reverse primers, and 3.5 µL of nuclease-free water in a total reaction volume of 10 µL. The qRT-PCR analysis was conducted via pre-PCR at 95 °C for 30 s, followed by 50 cycles of denaturation at 95 °C for 5 s, annealing at 59 °C for 10 s, and extension at 60 °C for 10 s.

Data analysis

The quantitative expression of the *LYZ-C* gene was determined on the basis of the relative expression of the *LYZ-C* gene in transgenic lines. Transgenic plants that had the lowest *LYZ-C* gene expression value were used as the standard and were given a value of 1 for *LYZ-C* gene expression. Relative gene expression was analyzed through the comparison of cycle of threshold (CT) as previously described (Livak and Schmittgen, 2001). Relative expression ($2^{-\Delta\Delta CT}$) was calculated by using the following formulas: $\Delta CT_{BC1F2} = CT_{LyzBC} - CT_{ActBC}$; $\Delta CT_{Std} = CT_{Std} - CT_{ActStd}$; and $\Delta\Delta CT = \Delta CT_{BC1F2} - \Delta CT_{Std}$. CT is the number of cycles required for the fluorescent signal to reach a certain threshold value. ΔCT is the difference in CT value between the *LYZ-C* gene and the actin gene. CT_{LyzBC} and CT_{LyzStd} are the CT values of the *LYZ-C* gene in the BC and standard plants, respectively. CT_{ActBC} and CT_{ActStd} are the CT values of the actin gene in the BC and standard plants, respectively. ΔΔCT is the difference between the ΔCT of BC1F2 plants and the ΔCT of the standard plants. The relative *LYZ-C* gene expression values between transgenic BC1F2 lines and standard plants were further analyzed by using Microsoft Excel 2010.

RESULTS

Segregation of the *LYZ-C* gene in F1 and BC1F1 generations

The crossing between 'Ciherang' and 'KinLys 1/3/23' produced 132 F1 generation seeds. Among the 132 F1 generation seeds, 43 were randomly germinated on selection medium containing hygromycin. All F1 plants and 'KinLys 1/3/23' were resistant to hygromycin, whereas 'Ciherang' was sensitive (Figure 1).

BC1F1 seeds were obtained from a backcross between wild-type 'Ciherang' and F1 generations carrying the *LYZ-C* gene. The segregation of the *LYZ-C* gene in the BC1F1 generation was determined on the basis of the inheritance of the *hpt* gene, which is linked to the *LYZ-C* gene, by germinating the seeds on MS medium containing 30 mg/L hygromycin for 3 weeks. The BC1F1 population was composed of two classes: one was resistant to hygromycin and exhibited healthy growth on the selection medium after 3 weeks, and the other was sensitive to hygromycin (Figure 2). From the 32 BC1F1 seeds germinated on selectionmedium, 18 were resistant to hygromycin and 14 were sensitive (Table 1). The χ^2 test showed that the BC1F1

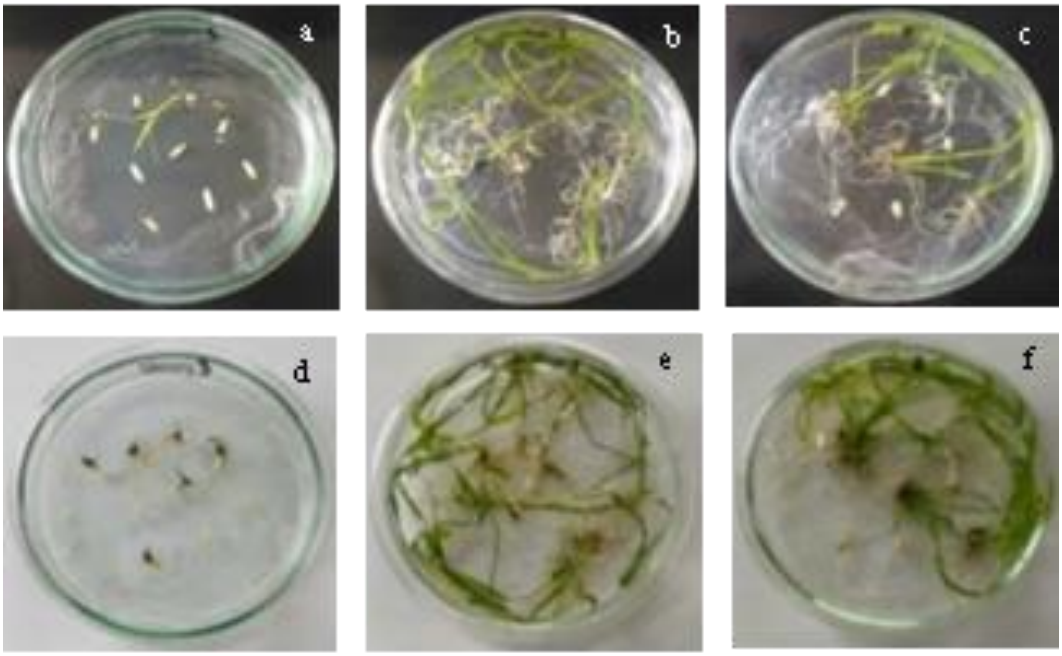

Figure 1. Hygromycin resistance analysis on selection medium. a–c = 2-week-old 'Ciherang', F1, and 'KinLys 1/3/23' seedlings; d–f = 4-week-old 'Ciherang', F1, and 'KinLys 1/3/23' seedlings.

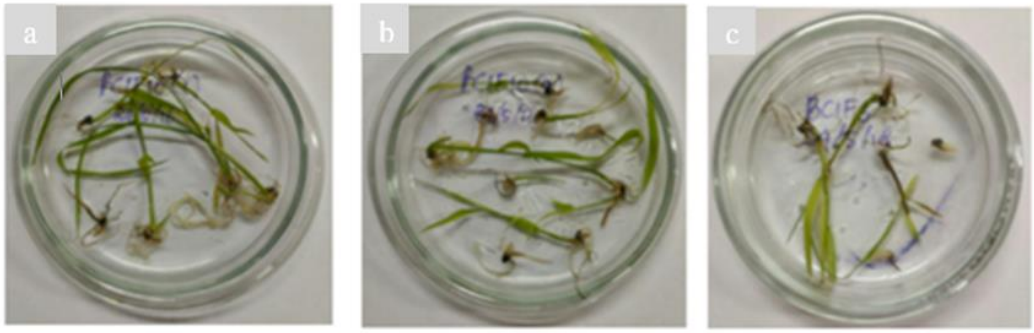

Figure 2. Analysis of the hygromycin resistance of the BC1F1 generation on selection medium.

Table 1. Segregation of the *hpt* gene linked to the *LYZ-C* gene in F1 and BC1F1 generations.

Transgenic generations	Total number of tested plants	Number of resistant plants	Number of sensitive plants	Resistant:sensitive	χ^2
F1	43	43	0	4:0	0
BC1F1	32	18	14	1:1	0.50

χ^2 table (with degree of freedom = 1 and α = 0.05) = 3.841

population segregated with a 1:1 ratio of hygromycin-resistant and hygromycin-sensitive plants in accordance with Mendel's law of segregation for a monohybrid dominant gene.

Analysis of *LYZ-C* gene inheritance

The integration of the *LYZ-C* gene in each generation was analyzed by performing PCR on all hygromycin-resistant plants. DNA was successfully extracted from 12 F1 plants. 'KinLys 1/3/23' and 'Ciherang' were used in PCR as positive and negative controls, respectively, to validate the PCR process. The PCR of the *LYZ-C* gene present in all the F1 generations and 'KinLys 1/3/23' resulted in a single 722 bp amplicon, whereas that in 'Ciherang' yielded no amplicon (Figure 3). This result indicated that the *LYZ-C* gene was successfully introduced from 'KinLys 1/3/23' into the F1 generation.

The PCR of the *LYZ-C* gene in one hygromycin-resistant plant of BC1F1 showed that this plant contained the *LYZ-C* gene as seen in Figure 4. The same PCR analysis as that conducted on F1 and BC1F1 generations was also conducted on BC1F2 generations. The results of the analysis showed that the *LYZ-C* gene was stably integrated into the genome of five plants of the BC1F2 generations (Figure 4).

Resistance of BC1F2 generations to *Xoo* pathotype IV

The resistance of the BC1F2 generations against BLB was evaluated by using *Xoo* pathotype IV, which is one of three dominant *Xoo* races in Indonesia (Suparyono *et al.,* 2004). The inoculation of *Xoo* was carried out on 50-day-old plants of five BC1F2 lines, 'Ciherang', and 'Kinlys1/3/23'. Observations obtained 14 DAI showed that the responses of the plants differed depending on the genotype. Necrotic areas in the leaves of 'Ciherang' were longer than those found in BC1F2 and 'KinLys 1/3/23' plants. 'KinLys 1/3/23', the DP, showed the lowest disease severity of 0.66%. This result indicated that it was highly resistant to *Xoo* pathotype IV. On the other hand, 'Ciherang' showed the highest disease severity at 28.22% and was classified as susceptible to *Xoo* pathotype IV.

Disease severity in BC1F2 plants varied from 0.77% to 9.33% (Table 2). The varied responses of BC1F2 plants to *Xoo* are illustrated in Figure 5. On the basis of the level of resistance to *Xoo* pathotype IV, the BC1F2 population was classified into three groups, i.e. one line as moderately resistant with disease severity indexes between 6%–12%, three lines as resistant with disease severity indexes between 1%–6%, and another line as highly resistant with disease severity indexes less than 1%. All BC1F2 plants showed higher resistance to *Xoo* pathotype IV than 'Ciherang' (Table 2). The enhanced resistance of BC1F2 plants compared with that of 'Ciherang' indicated that *LYZ-C* transgene was successfully expressed in BC1F2 plants and played a role in increasing plant resistance to BLB. The lysozyme gene from transgenic *japonica* rice has also been successfully transferred into the parents of hybrid rice to develop hybrid rice with resistance to rice blast (Yi *et al.,* 2006).

Expression of the *LYZ-C* gene in BC1F2

PCR amplification by using a primer pair for the actin gene located at exons 2 and 3 of the rice actin gene amplified 377 bp of its cDNA and 492 bp of genomic DNA of the part of actin gene. Given that the region between exons 2 and 3 of the actin gene was separated by intron 2, the size of the cDNA was approximately 115 bp smaller than that of genomic DNA. The cDNA of 'Ciherang', 'KinLys 1/3/23', and five plants of BC1F2 was not contaminated by genomic DNA (Figure 6).

The expression of the *LYZ-C* gene in BC1F2 plants was qualitatively analyzed via PCR by using Lys-F and Lys 150-F primers. All BC1F2 plants and the parent 'KinLys 1/3/23' expressed the *LYZ-C* gene, whereas 'Ciherang' did not express this gene (Figure 7).

Table 2. Disease severity of BLB and level of resistance to BLB.

Genotypes	Response to *Xoo* pathotype IV	
	Disease severity (%)	Resistance level
'Ciherang'	28.22	S
'KinLys 1/3/23'	0.66	HR
BC1F2-7	1.30	R
BC1F2-8	1.11	R
BC1F2-9	9.33	MR
BC1F2-10	1.97	R
BC1F2-11	0.77	HR

S: susceptible; R: resistant; MR: moderately resistant; HR: highly resistant

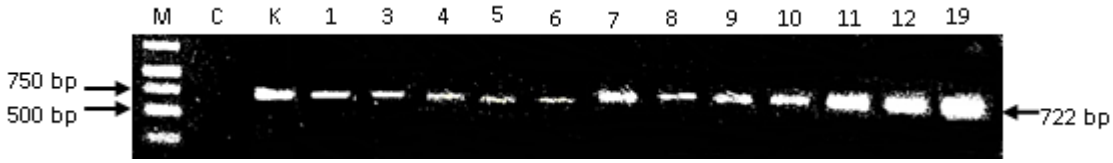

Figure 3. PCR amplification of the *LYZ-C*gene in the F1 population. M = 1 kb ladder; C= 'Ciherang'; K= 'KinLys 1/3/23'; 1 = F1–1; 3 = F1–3; 4 = F1–4; 5 = F1–5; 6 = F1–6; 7 = F1–7; 8 = F1–8; 9 = F1–9; 10 = F1–10; 11 = F1–11; 1 2= F1–12; 19 = F1–19.

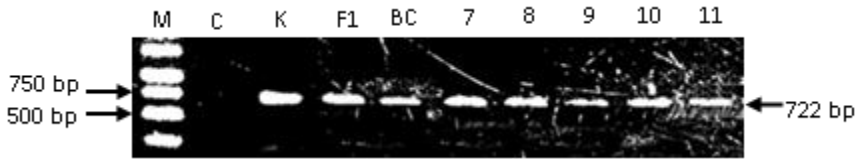

Figure 4. PCR amplification of the *LYZ-C* gene in the BCF1 and BC1F2 populations. M = 1 kb DNA ladder; C = 'Ciherang'; K = 'KinLys 1/3/23'; F1 = F1 plant; BC = BC1F1–12; 7 = BC1F2–7; 8 = BC1F2–8; 9 = BC1F2–9; 10 = BC1F2–10; 11 = BC1F2–11

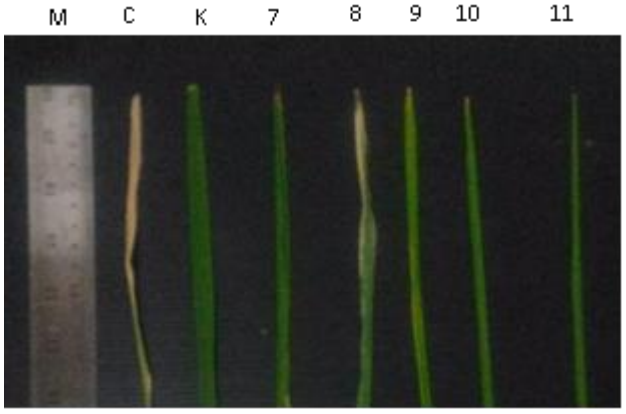

Figure 5. Variation in the severity of *Xoo* pathotype IV infection in the BC1F2 population 14 DAI. M = marker; C = 'Ciherang'; K = 'KinLys 1/3/23'; 7 = BC1F2–7; 8 = BC1F2–8; 9 = BC1F2–9; 10 = BC1F2–10; 11= BC1F2–11.

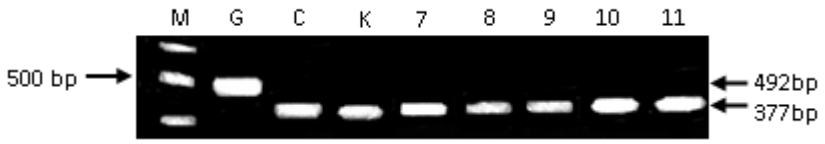

Figure 6. PCR amplification of the genomic DNA and cDNA of the actin gene in the BC1F2 population and its parents. M = 1 kb DNA ladder; G = 'Ciherang' genomic DNA; C = 'Ciherang' cDNA; K = 'KinLys 1/3/23' cDNA; 7 = BC1F2–7 cDNA; 8 = BC1F2–8 cDNA; 9 = BC1F2–9 cDNA; 10 = BC1F2–10 cDNA; 11 = BC1F2–11 cDNA.

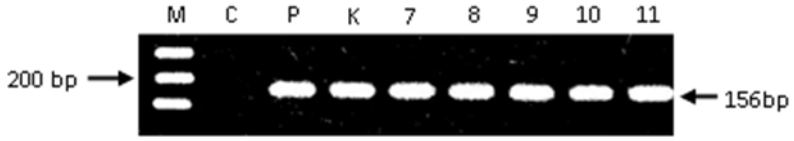

Figure 7. PCR analysis of *LYZ-C* gene expression in BC1F2. M = 100 bp DNA ladder; C = 'Ciherang'; P = pMSH-Lys; K = 'KinLys 1/3/23'; 7 = BC1F2–7; 8 = BC1F2–8; 9 = BC1F2–9; 10 = BC1F2–10; 11 = BC1F2–11.

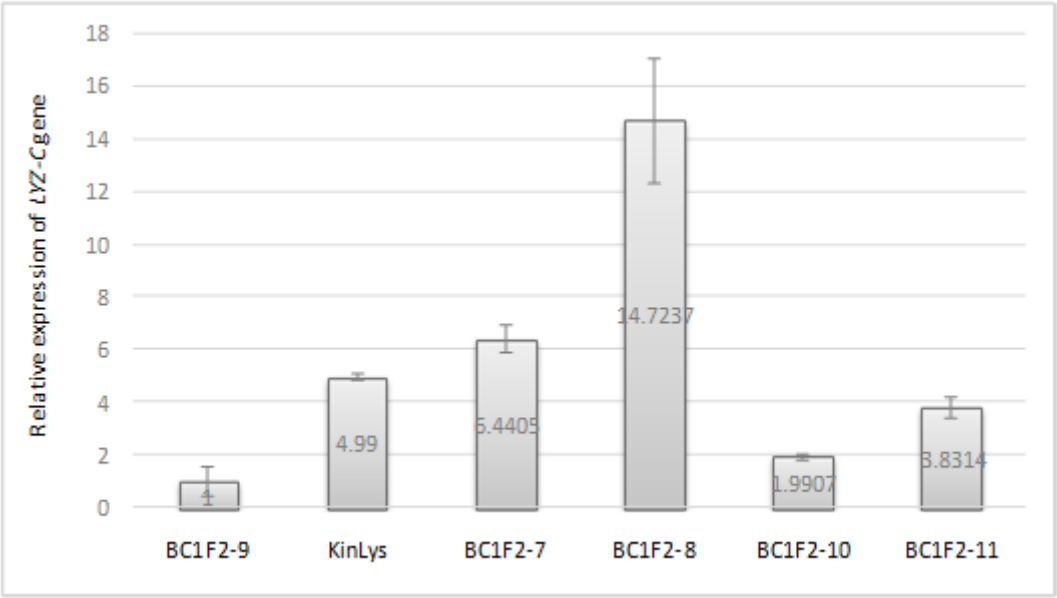

Figure 8. Quantitative expression of the *LYZ-C* gene in the BC1F2 population compared with that in BC1F2-9. The values represented are an average of three independent sets of experiments. Bars represent the mean standard error of three independent sets of experiments.

Quantitative expression analysis by qRT-PCR showed variations in the expression levels of the *LYZ-C* gene among BC1F2 plants and 'KinLys 1/3/23'. Quantitative expression analysis showed that the BC1F2-9 plants had the lowest *LYZ-C* gene expression level. Therefore, these plants were used as the standards for analysis. *LYZ-C* gene expression in the BC1F2-8 line was highest among that in all BC1F2 lines and 'KinLys 1/3/23' (Figure 8).

DISCUSSION

Introgression of the *LYZ-C* gene into 'Ciherang' rice

In conventional breeding, the R gene is crucial for increasing the resistance of rice against *Xoo*. At least 43 resistance genes exist, and dominant and recessive resistance genes have been identified (Vikal and Bhatia, 2017; Kim and Reinke, 2019). Resistant rice cultivars found in nature generally have a single resistance gene. Rice cultivars with broad-spectrum and increased *Xoo* resistance are obtained by pyramiding lines with two or more BLB resistance genes. However, the resistance properties of the pyramided lines are easily broken down by the capability of *Xoo* to form new strains with enhanced virulence in a relatively short time (Vera-Cruz *et al*., 2000; Ochiai *et al*., 2005).

Another source of resistance genes is needed to develop a rice cultivar with high broad-spectrum resistance. C-type lysozyme can degrade the β-1, 4 glycoside bond between N-acetylmuramic acid and N-acetylglucosamine in bacterial cell walls. It can be used as a new resistance source. However, the *LYZ-C* gene is not originally found in the rice genome and therefore cannot be transferred into the rice genome through conventional breeding. These limitations can be addressed through genetic engineering to avoid the transsexual barrier present across biota and combine transgenes from heterologous sources. Therefore, we combined a genetic engineering technique and a conventional breeding technique to accelerate the breeding process for a new variety of *indica* rice with transgenic characters.

In this study, we used the transgenic *japonica* rice 'KinLys 1/3/23' as the donor of the *LYZ-C* gene and 'Ciherang' as the RP. The *LYZ*-C gene was successfully transferred into the elite *indica* rice parents by crossing followed by backcrossing. Crossing between 'Ciherang' and 'KinLys 1/3/23' provided F1 plants that were all transgenic.

Backcrossing was carried out by crossing one randomly selected F1 plant and one 'Ciherang' plant as the RP to generate the F1BC1 population to simplify the introgression. The F1BC1 population was composed of transgenic and nontransgenic plants at a 1:1 ratio. This result indicated that the transgenes were inherited in a Mendelian pattern with one copy of transgenes integrated into the transgenic plant. One plant that was selected randomly from the transgenic BC1F1 population was self-pollinated to produce the BC1F2 population. Analyzing the five randomly selected hygromycin-resistant BC1F2 plants showed that these plants also carried the *LYZ-C* gene. This result showed that the transgenes were stably integrated into the genome of the BC1F2 generation and that the *hpt* gene was linked to the *LYZ-C* gene as expected.

Factors that affected the resistance of BC1F2 plants to BLB

BLB can be damaging to rice at all growth stages. It is affected by environmental conditions, the pathogenicity of the pathogen, the distribution of varieties, and other factors. We inoculated the same strain of *Xoo,* which is one of the most dominant *Xoo* races with strong pathogenicity in Indonesia, into five randomly selected transgenic BC1F2 plants to identify BLB resistance in the BC1F2 population. All BC1F2 plants and 'KinLys 1/3/23' as DPs were more resistant to BLB than 'Ciherang'.

Notably 'KinLys 1/3/23' line was more resistant to BLB than the offspring obtained through backcrossing, i.e., the BC1F2 population. 'KinLys 1/3/23' was homozygous for the *Lyz-C* gene as indicated by the observation that all F1 population lines were resistant to hygromycin, whereas the BC1F2 population was composed of mostly hemizygous plants and a small proportion of plants that were homozygous for the *LYZ-C* gene. Therefore, four out of five lines of the BC1F2 population, i.e., BC1F2-7, BC1F2-8, BC1F2-9, and BC1F2-10, were less resistant to BLB than 'KinLys

1/3/23'. Only the BC1F2-11 line had the same level of resistance to BLB as 'KinLys 1/3/23'. Similar to 'KinLys 1/3/23', BC1F2-11 might have a homozygous genotype for the *LYZ-C* gene because it had the same level of resistance to BLB. The contribution of the genome of the parents to each individual from the F2 population was different such that the individuals had different levels of resistance to BLB. In addition to the *LYZ-C* gene, other genes present in F2 individuals originating from their parents affected resistance to BLB. Interaction between the *LYZ-C* gene and other genes can affect plant resistance to BLB. In this study, the BC1F2 population showed three levels of resistance to BLB: moderate resistance, resistance, and high resistance. The different BLB resistance levels shown by the BC1F2 population might be due to the level of homozygosity of *the LYZ-C* gene, wherein *LYZ-C* homozygous plants were more resistant than hemizygous ones. Furthermore, we suspected that BC1F2-11 was *LYZ-C* homozygous genotype and the other BC1F2 lines were hemizygous genotypes. Further investigation is needed to identify the reason for this phenomenon. The resistance of rice plants to BLB disease was related to the expression of the *LYZ*-C gene as indicated in Figure 7. All transgenic BC1F2 plants expressed the *LYZ-C* gene, whereas 'Ciherang' rice did not express this gene. Therefore, all transgenic plants were more resistant than 'Ciherang' rice.

Quantitative *LYZ-C* gene expression in the BC1F2 population varied. Variation in the level of expression of the *LYZ-C* gene in the BC1F2 population was thought to be caused by interaction between several genes of DPs and recipient parents. Gene interaction, including the interaction between encoded proteins, can increase or decrease gene expression. In some BC1F2 generations, this interaction had an effect on the increased expression of the *LYZ-C* gene beyond expression in the DP 'KinLys 1/3/23'. However, the level of *LYZ-C* gene expression in this generation, except in BC1F2-9, was not related directly to the BLB resistance level. The *LYZ-C* gene expression in BC1F2-9 plants was lowest among that in all transgenic plants and was correlated with plant resistance to BLB disease, wherein BC1F2-9 had the lowest resistance. The highest expression level of the *LYZ-C* gene in other transgenic plants was not directly correlated to the highest BLB resistance level as can be seen in the BC1F2-8 and BC1F2-7 lines. The expression levels of the *LYZ-C* gene in these two lines were 14.7 and 6.4 times higher than those in BC1F2-9, respectively. However, these lines were classified only as resistant to *Xoo* pathotype IV. By contrast, although the *LYZ-C* gene expression level in the BC1F2-11 line was 3.8 times higher than that in BC1F2-9, this line was considered highly resistant to *Xoo* pathotype IV.

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