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INTROGRESSION OF RESISTANCE TO BLAST DISEASE FROM MONOGENIC LINE IRBLta2-Re TO CIHERANG RICE VARIETY

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SUMMARY

Rice (*Oryza sativa* L.) cultivar Ciherang is most widely cultivated in Indonesia since it has a high yield and resistance to some pests and diseases. Nevertheless, in recent years, there has been a decline in the resistance trait of Ciherang to blast disease. Therefore, the efforts to improve the resistance trait of Ciherang to blast disease are needed. The improvement of Ciherang resistant trait can be done by introgression of a resistance gene from IRBLta2-Re monogenic line (ML) carrying *Pita-2* gene. IRBLta2-Re has a broad spectrum of resistance to blast. The objective of this study was to introgress the blast-resistant trait from the IRBLta2-Re to the Ciherang cultivar. The research began with a cross between Ciherang as the female parents and IRBLta2-Re as the male parents to create an F_1 population. This F_1 population was then crossed to Ciherang as recurrent parent to create a backcross population. *Pyricularia oryzae* race ID96 was used to select the blast-resistant trait in populations BC_1F_1, BC_2F_1, and BC_3F_1. Selection to 2074 plants of BC_1F_1 population resulted in 148 plants resistant to blast disease with a 0 score. The BC_2F_1 population was developed by backcrossing four selected blast-resistant BC_1F_1 lines *viz*. lines 627, 1141, 2129 and 2192. Screening to 848 plants of the BC_2F_1 population showed that 59 plants were blast-resistant with a 0 score. Among 59 blast resistant lines of BC_2F_1 population, two lines, i.e. lines 627-5 and 2192-3 were separately crossed with recurrent parent to develop BC_3F_1. Screening to 244 plants of the BC_3F_1 population resulted in 32 plants resistant to blast disease. These results indicate that introgression of the *Pita-2* gene from IRBLta2-Re to Ciherang was successful and produced blast-resistant lines in the BC_1F_1, BC_2F_1 and BC_3F_1 populations.

Key words: Blast disease, Ciherang, resistance, rice, selection

Key findings: *Pita-2* blast resistant gene was succesfully introgressed from IRBLta2-Re rice into rice var. Ciherang. Several BC_3F_1 lines resistant to blast disease with agronomic characters similar to Ciherang had been obtained.

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INTRODUCTION

The efforts to increase rice (*Oryza sativa* L.) production are constantly being challenged by various problems and obstacles on abiotic and biotic factors. Diseases are a limiting factor in cultivation and cause productivity to decrease. Diseases those commonly attack rice plants on the cultivation fields could be caused by viruses, mycoplasma-like organisms (MLO), bacteria, fungi, nematodes, and physiological disorders (Ou, 1985). The dynamics of disease of rice vary depending on the different planting time and planting season (Yuliani *et al.,* 2017).

Blast is the most globally widespread disease in rice. It is the main disease of all cereals in the world and is very destructive in the conducive environment for *Pyricularia* spp*.* (Scardaci *et al.,* 1997; Talbot, 2003). In Indonesia, blast disease was initially the main issue in upland rice; however, over time, the disease also began to attack tidal swamp rice and lowland rice varieties. A number of techniques have been applied to control the blast disease, and each technique has its advantages and disadvantages. The use of blast disease-resistant varieties is the most effective, affordable, and environmentally-friendly method for controlling the blast disease (Toha, 2007; Fukuta *et al.,* 2009; Yulianto, 2017).

The creation of blast-resistant varieties can be done through introgression of the desired gene from the donor parents to the recipient parents using the backcross method (Allard, 1999). The backcross (BC) method is widely used in rice breeding to create blast-resistant varieties (Divya *et al.,* 2014; Lee *et al.,* 2015; Ellur *et al.,* 2016; Khan *et al.,* 2018). The BC method could also recover the recipient parent's genome by eliminating unwanted genes (linkage drag) (Xi *et al.,* 2008; Hasan *et al.,* 2015). This method can be used to improve varieties having good agronomic traits and adaptation but lacking one or more traits.

Ciherang is an Indonesian cultivar of rice which has a high potential yield with 7-8 ton/hectare. It produces long and slender grains with clean yellow color. The texture of the cooked rice is soft rather sticky. Ciherang is most widely cultivated by farmers in Indonesia and is very popular among farmers and consumers. About 30.44% of the rice area in Indonesia is cultivated with Ciherang. Ciherang has resistance to some pests and diseases such as the rice brown leafhoppers biotype 2 and 3, and bacterial leaf blight strain III and IV. It is resistant to some races of *Pyricularia oryzae*, a causal agent of blast disease. Nevertheless, in recent years, there has been a decline in the resistance trait of Ciherang to blast disease. Therefore, the efforts to improve the resistance trait of Ciherang to blast disease are needed.

Eventhough Ciherang is resistant to some races of *P. Oryzae,* recently it was reported to be susceptible to certain blast race as ID96 (Fitriah *et al*., 2019). To overcome the blast disease caused by new race of *P. oryzae*, as ID96, and to broaden the resistance spectrum, the blast resistant gene carried by IRBLta2-Re monogenic lines was introgressed into the genome of Ciherang rice.

IRBLta2-Re carrying *Pita-2* gene has a broad spectrum of blast resistance to Philippines and Chinese isolates (Kobayashi *et al.,* 2006; Lei, 2014). This line has also a broad spectrum of blast resistance caused by Indonesia isolates (Suwarno *et. al.,* 2014; Fitriah *et al.,* 2019). It is highly resistant to *P. oryzae* race ID96, whereas Ciherang is very susceptible to this race. This is the reason that IRBLta2-Re monogenic line was chosen as a donor of blast resistant gene to improve and to broaden the blast resistance of Ciherang rice. Therefore, the aim of this research was to introgress pita-2 blast resistant gene carried by IRBLta2-Re line into the genome of Ciherang rice by backcrossing.

MATERIALS AND METHODS

IRBLta2-Re rice (*O. sativa* L.) was used as *Pita-2* gene donor parents and Ciherang rice as the recipient (recurrent) parents. *P. oryzae* race ID96 was used to screen the resistant rice to blast disease. Race ID96 was isolated lowland rice in Indonesia. Based on Indonesia differential variety screening, ID96 is same to race 173. Rice var. Kencana Bali was used as a control susceptible variety to blast disease to confirm that innoculation was succesfully done.

Ciherang rice was pollinated by the donor IRBLta2-Re at Muara Experimental Station, Indonesian Center for Rice Research, Indonesian Agency of Agricultural Research and Development, Bogor, Indonesia. The F_1 plants were backcrossed to Ciherang as a recurrent parent to obtain BC_1F_1 seeds. Of the 3000 BC_1F_1 seeds, only 2074 plants were screened. The BC_2F_1 populations were obtained from the crossing of Ciherang with four selected blast resistant plants of BC_1F_1 population, i.e. line 627, line 1141, line 2129 and line 2192. Two selected blast resistant plants of BC_2F_1 population, namely line 627-5 and line 2192-3, were used to pollinate Ciherang rice to obtain BC_3F_1 population.

Selection of resistance to blast disease in the BC_1F_1, BC_2F_1, and BC_3F_1 populations was conducted using the method of Hayashi *et al.* (2009) and carried out in a greenhouse. Observations and scoring of the blast disease severity rate were based on the IRRI Evaluation Standard System (IRRI, 2014), with a 0 severity index for the highly resistant plant criterion, 1-3 for resistant, 4-6 for moderately resistant or moderately susceptible, and 7-9 for susceptible plants.

RESULTS

Introgression of the blast-resistant trait from IRBLta2-Re into Ciherang in backcross populations

The crossing between Ciherang and IRBLta2-Re produced 230 F_1 seeds and all of these seeds were planted to create the BC_1F_1 population. The agronomic characters of F_1 plants were a combination of the two parents. The agronomic characters of the F_1 plants are presented in Figure 1.

Most of F_1 plants had the similar height as the Ciherang parent, around 80-100 cm. The number of productive tillers of the F_1 was greater than that of the IRBLta2-Re parent, around 5-8 tillers. The leaf surface of F_1 plants is rough in texture; the plant shape and leaves are upright; the flag leaf of F_1 plant's is erect similar to the Ciherang parent. The F_1 plants had an earlier flowering age than Ciherang, around 95-105 days. The size and shape of the F_1 plant's grain were medium and fairly rounded, unlike both of the parents. The grain color of F_1 plant's is yellow. In general, the F_1 plant has combination characters of both parents. Selection of resistance to *P. oryzae* was not conducted on the F_1 plants.

The crossing between F_1 population and Ciherang as female parent produced about 3000 BC_1F_1 seeds, but only 2074 plants grew well enough to be screened. Screening to obtain the resistant plants to blast disease by using *P. oryzae* race ID96, resulted 148 resistant plants at score 0 and 1209 plants at score 1. There were 680 plants that were moderately resistant (score 3) and 37 plants that were susceptible at score 5. These result showed that BC_1F_1 population was composed of 98% resistant plants (dominant trait) and 2% susceptible plants (recessive trait). We did not find the highly susceptible plants with score 7 or more. The distribution of the blast-resistant trait in the BC_1F_1 population is presented in Figure 2.

In general, the agronomic characters and phenotype of the resistant BC_1F_1 plants had a similarity to the Ciherang parent (Table 1). The BC_1F_1 plants had almost the same height as Ciherang parents, even though some BC_1F_1 plants had a taller than the Ciherang. Plant shape and leaf of BC_1F_1 plants were similar to that of Ciherang. The flowering time for the BC_1F_1 plants was nearly uniform and was almost the same as Ciherang, eventhough there were 10 plants of BC_1F_1 population that had an earlier flowering time at ±83-105 days. The BC_1F_1 grain color and shape were principally the same as Ciherang but they were slightly rounded, different than Ciherang grain which is slender long.

From the 148 BC_1F_1 resistant plants with score 0, 69 plants were similar to Ciherang. Among 69 BC_1F_1 plants, four plants were selected to be back-crossed to Ciherang to obtained BC_2F_1 population. The selected BC_1F_1 lines are line 627, line 1141, line 2129 and line 2192. The criteria of selection of resistant BC_1F_1 were based on the similarity to Ciherang characters covering plant height, plant shape, position of flag leaves, form and size of grain, and molecular marker for *Pita-2* gene and Ciherang genome segment (Table 2). The characters of selected BC_1F_1 resistant to *P. oryzae* race ID96 are presented in Table 3.

The BC_2F_1 population was obtained by back crossing four selected BC_1F_1 plants with the Ciherang as female parents. Among 796 BC_2F_1 plants, 140 plants were resistant to blast disease with score 1, 479 plants with score 3, 54 plants and 23 plants were moderately susceptible with score 5 and susceptible with

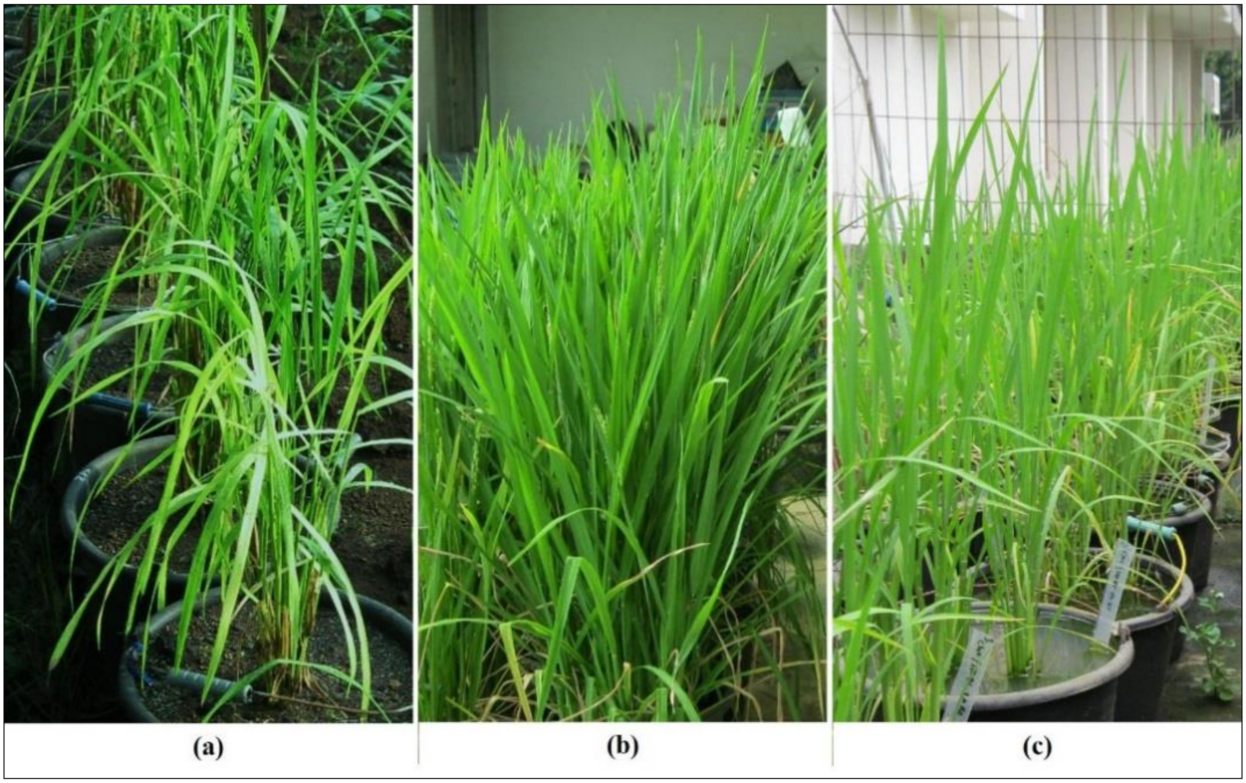

Figure 1. Agronomic characters of F_1 population. (a) IRBLta2-Re rice (donor), (b) Ciherang rice (recipient) and (c) F_1 plants.

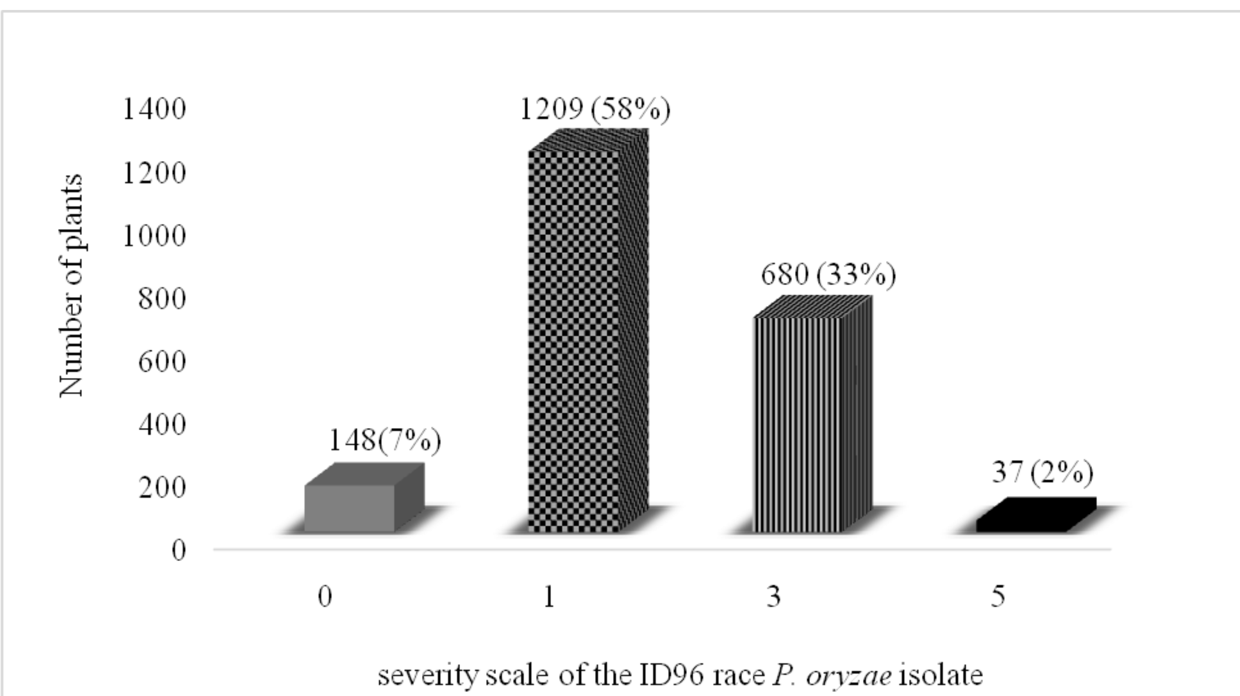

Figure 2. Distribution of resistance to the ID96 race *P. oryzae* in BC_1F_1.

Table 1. General traits of rice BC_1 plant population.

Trait	Cross		BC1 plant
	Ciherang	F_1	
Grain shape	Long	Medium, fairly rounded	Medium fairly long
Color of stem	Green	Green	Green
Plant shape	Upright	Upright	Upright
Flag leaf	Erect	Erect	Erect
Grain color	Clean yellow	Yellow	Yellow
Blast-resistance	Susceptible	Resistant	Resistant

Table 2. The genotypes confirmation in the selected BC_1F_1.

Selected BC_1F_1 plants	Genotype confirmation							
	a	b	c	d	E	f	g	H
627	+	+	+	+	+	+	+	+
1141	+	-	+	+	+	+	+	+
2129	+	+	+	+	+	+	-	+
2192	+	+	+	+	+	+	-	+

(+) = amplicon; (-)= no amplicon; a-h = primers used to detect of *Pita-2* gene and Ciherang segment; a) RM7102; b) ta801-1F; c) ta577-1F; d) RM247; e) RM101; f) SNP-Ciherang-1-4; g) SNP-Ciherang-12-2-2; h) SNP-Ciherang-12-3-2.

Table 3. Selection for resistance to ID96 race *P. oryzae* in the BC_2F_1 population.

No. BC_1F_1 line	Σ BC_2F_1 plant	*P. oryzae* severity scale			
		1	3	5	7
627	297	99	167	31	0
1141	163	11	100	47	5
2192	283	19	173	74	17
2129	53	11	39	2	1
Total	796	140	479	154	23

score 7, respectively. Therefore, 796 BC_2F_1 plants can be classified into two groups, 619 plants are resistant and 77 susceptible plants to *P. oryzae* race ID96.

In general, the agronomic characters as plant shape, stem color, flag leaf position, number of productive tillers, shape and color of grains of the ID96 resistant plants of BC_2F_1 population were similar to Ciherang parent (Table 4, Figure 3). However, the grains of BC_2F_1 plants were not as slender as Ciherang grains but the color of grains is clean yellow as Ciherang grains and different than IRBLta-Re grains.

From 140 ID96 resistant plants of BC_2F_1 population, two plants i.e. line 627-5 and line 2192-3 were selected to pollinate Ciherang to develop BC_3F_1 population. These two BC_2F_1 plants had a score-1 resistance to *P. oryzae* race ID96 and agronomic characters 90% similar to those of the Ciherang.

From 244 plants of BC_3F_1 population, 131 plants were considered as resistant and 113 plants were susceptible (Table 5). Based on

Table 4. General traits of the ID96 resistant plants in rice BC_2F_1 population.

Trait	Cross		BC_2F_1 Plants
	Ciherang	BC_1F_1	
Grain shape	Long	Medium fairly long	Long
Color of stem	Green	Green	Green
Plant shape	Upright	Upright	Upright
Flag leaf	Erect	Erect	Erect
Grain color	Clean yellow	Yellow	Clean yellow
Blast-resistance	Susceptible	Resistant	Resistant

Figure 3. Morphology of grains of selected BC_2F_1. (a) grains of Ciherang , (b) grains of IRBLta2-Re, (c) grains of BC_2F_1-627-5, (d) grains of BC_2F_1-2192-3.

Table 5. Selection of the resistance to the ID96 race *P. oryzae* in the BC_3F_1 population.

No. BC_2F_1 line	Σ BC_3F_1 plant	*P. oryzae* severity scale			
		1	3	5	7
627-5	139	14	62	61	2
2192-3	105	18	37	49	1
Total	244	32	99	110	3

Table 6. The genotypes confirmation in the selected BC_2F_1.

Selected BC_1F_1 plants	Σ Selected plants	Σ BC_2F_1 plants confirmed by genotype						
		a	b	c	d	e	f	g
627	18	9	13	14	8	17	17	13
1141	11	8	4	8	4	9	11	10
2129	11	8	11	10	3	11	11	11
2192	19	17	8	9	14	19	19	18

a-h = primers used to detect of *Pita-2* gene and Ciherang segment; a) RM7102; b) RM28009; c) ta801; d) ta577; e) SNP-Ciherang-1-4; f) SNP-Ciherang-12-2-2; g) SNP-Ciherang-12-3-2.

the resistance to *P. oryzae* race ID96, the BC_3F_1 population was composed of 53.68% resistant and 46.31% susceptible plants. Based on qualitative observation, the morphology of the BC_3F_1 plants which had the resistance to the ID96 race *P. Oryzae* was ≥ 90% similar to those of the Ciherang (Table 6). The agronomic character of BC_3F_1 is uniform, the height of plant ranged ± 107-115 cm similar to Ciherang. The plant shape and position of flag leaves was upright and erect like Ciherang. The number of tillers is around ± 14-20. The flowering age was uniform and similar to Ciherang which ranges ±116-125 days. The color and shape grains of BC_3F_1 resistant are clean yellow and slim similar to Ciherang.

DISCUSSION

The IRBLta2-Re is a monogenic blast line from IRRI, which brings the *Pita-2* gene from the donor parent Reiho. IRBLta2-Re has a broad spectrum of resistance traits (Kobayashi *et al.,* 2006; Lei *et al.,* 2014). We used IRBLta2-Re to improve blast resistance of Ciherang. The improvement of Ciherang can be done by introgression, and this method is fit to fix one or more traits in a variety. Since Ciherang already has resistance to several races of *P. oryzae* (Fitriah *et al*., 2019), we expected that this resistance could be broadened to other races by crossing with IRBLta2-Re.

The traits of F_1 population of crossing between Ciherang and IRBLta-Re have shown the combination of its parents. The plant height of F_1 population is similar as the Ciherang parent, around 80-100 cm. It means that plant height trait is dominant. The productive tiller number trait carried by IRBLta2-Re parent appears to be recessive trait since the F_1 population had the productive tiller number more than that of IRBLta2-Re. The trait of leaf surface, plant shape, upright leaves, the shape of flag leaf of Ciherang appears to be the dominant traits. Flowering time of F_1 population is earlier than Ciherang, indicating that flowering time of Ciherang is recessive trait. The size, shape and color of grains of the F_1 are different than those parents, indicating that these traits are codominant. Therefore, F_1 population has a combination of characters of its parents, Ciherang and IRBLta-Re.

The resistant and susceptible ratio was not 3:1 or 1:1 in BC_1F_1 population because Ciherang is not completely succeptible to blast disease. Previous research showed that Ciherang is resistant to several races of *P. oryzae* (Suwarno *et al.* 2014; Fitriah *et al.* 2019), therefore we assume that Ciherang carries some resistant genes and these genes contibute to the high proportion of resistant plants to blast disease. Other possibility is that the resistance to race ID96 is controlled by more than two alleles of *Pita-2* gene and the resistant allele (R) is dominant.

Based on the resistance to blast disease, the BC_1F_1 population was composed of three classes, as resistant with score 0 and 1, moderate resistant with score 3 and susceptible with score 5. If moderate resistant is considered as resistant class, the BC_1F_1 population was composed of 98% resistant plants with R-genotypes (dominant trait) and 2% susceptible plants with the rr_n genotype (recessive trait). F_1 is heterozygous for the resistant trait (R_nr_n), and Ciherang as recurrent parents is homozygous for susceptible trait (r_nr_n), where n is number of alleles. If the resistance to race ID96 is determined by single gene, the BC_1F_1 population will have the proportion of 50% resistant (Rr) and 50% susceptible (rr) to *P. oryzae* ID96, and if the resistance is controlled by two alleles, the frequency of resistant plants in BC_1F_1 is 75% (R_1--- and R_2---). The frequency of resistant plants in BC_1F_1 population is 96.3 %, if the resistant trait is controlled by three alleles. In fact, the resistance to *P. oryzae* in this BC_1F_1 population was 98%, therefore the ID96 resistant trait is controlled by four alleles. By four alleles, the resistant plants in BC_1F_1 have a genotype (R-------) and the genotype of susceptible plants is $r_1r_1r_2r_2r_3r_3r_4r_4$ and the frequency of resistant plants is 98% and susceptible plants is 2%. By this result, we predict that IRBLta2-Re line has *Pita-2* gene composed of four alleles. This *Pita-2* gene is the dominant gene that has a complete resistance reaction (Kobayashi *et al.,* 2006; Lei *et al.,* 2014; Suwarno *et al.,* 2014). In the other side, Ciherang also has a resistant gene to several races of *P. oryzae* as race 033, 073, 133, 173, ID36, dc4 and 001 (Fitriah *et al*., 2019). One of Ciherang's parents was IR64, which has blast resistance genes (Sallaud *et al*., 2003). These resistant genes carried by Ciherang and multiple resistant alleles carried by IRBLta-Re can contribute to the high frequency of blast resistant in BC_1F_1 population.

Based on the severity score (IRRI, 2014), the resistance of the BC population to *P. Oryzae* race ID96 can be classified into four groups as very resistant, resistant, moderate resistant or moderate susceptible and susceptible. The susceptible plants with a 7-9 score were not found in BC_1F_1 but they were found in BC_2F_1 and BC_3F_1. The frequency of resistant plant in BC_2F_1 and BC_3F_1 decreased from 98% in BC_1F_1 to 77 % in BC_2F_1 and 54 % in BC_3F_1. The decrease of resistance in BC population may be due to the loss of resistant alleles carried by IRBLta-Re (R allele) and the addition of susceptible allele (r) from Ciherang. Therefore, the resistance to race ID96 of BC population may be controlled by several alleles of *Pita-2* gene in IRBLta-Re.

The blast-resistant trait was successfully introgressed from IRBLta2-Re into the genome of Ciherang cultivar. Ciherang-like BC_3F_1 lines which have resistance to rice blast disease have been obtained and can be used to create an essential derivative varieties after eliminating unnecessary traits of IRBLta-Re by several back crossings.

The genome segregation in the BC population follows Mendel's law. The backcross method could recover the recipient parent's genome (recurrent) by eliminating the unwanted genes or segments from the donor (linkage drag) (Hasan *et al.,* 2015). Backcrossing is a method that can be used to improve a variety that has good agronomic traits but has one or more undesirable traits. This method is very suitable for the improvement of variety traits which are controlled by dominant genes. The backcross method can be applied in the development of NIL (near-isogenic line) lines which could then be used in the development of multiline varieties (Suwarno *et al*., 2001). Theoretically, the genome of BC_1F_1 population should be composed of 75% Ciherang genome, and 25% IRBLta-Re genome, BC_2F_1 should composed of 87.5% Ciherang and 12.5% IRBLta-Re, and BC_3F_1 genome should be composed of 93.25% Ciherang genome and 6.75% IRBLta-Re genome. To eliminate the genome of IRBLta-Re, except *Pita-2* gene, the back cross has to be continued. We assume that the frequency of recurrent genome, in BC_nF_1 generation is 100%-(50%)$^{n+1}$. At sixth generation of back cross (BC_6F_1) we will get population containing 99.2% Ciherang genome. By selfing of BC_6F_1 in several generation, the stable homozygous Ciherang like cultivar with broad spectum of blast resistance will be obtained.

CONCLUSION

The introgression of *Pita-2* gene responsible to the resistance to *P. oryzae* race ID96 of IRBL-Re to the Ciherang variety was successful. Actually, we obtained BC_3F_1 lines similar to Ciherang with additional blast resistance. The selected BC_3F_1 line can be used to create an essential derivative varieties.

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