INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0379/2019/22–3–468–474
DOI: 10.17957/IJAB/15.1088
http://www.fspublishers.org

Full Length Article

Screening of Markers and Blast Isolates for Blast Resistance Selection on Backcross-Population from Crossing Ciherang and IRBLta2-Re

Nurul Fitriah[1*], Utut Widyastuti[2,3†], Suharsono[2,3*], Satya Nugroho[4], Suwarno[5] and Miftahudin[2]
[1]*Graduate Program in Plant Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor 16680, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor 16680, Indonesia*
[3]*Research Center for Bioresources and Biotechnology, Bogor Agricultural University, Bogor 16680, Indonesia*
[4]*Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong, Indonesia*
[5]*Indonesian Center for Rice Research, Bogor, Indonesia*
[*]For correspondence: sony.suharsono@yahoo.com; nurulfitriah2@gmail.com

Abstract

Blast disease caused by fungal *Pyricularia grisea* Sacc. is one of the extremely damaging diseases that affect paddy. The development of resistant varieties has been proved to be a promising prevention method. Blast R genes are predictedto have played an essential role in paddy plants' defense responses. *Pita-2* gene carried by IRBLta2-Re, which is japonica paddy used as a differential variety for blast resistance. Ciherang is one of the Indonesian varieties with high yield but it is susceptible to blast disease. We are using marker assisted backcrossing selection approach to develop new Ciherang with a blast-resistant *Pita-2* gene. The research aims to get the standard blast isolates and *Pita-2* markers for blast screening on backcross-population of Ciherang and IRBLta2-Re. Screening on eight blast races and 10 paddy varieties showed that the ID96 race has a low virulency on IRBLta2-Re and high virulency on Ciherang with the intensity of the disease around 3% to 37%. Molecular analysis using 30 markers displayed 15 polymorphic markers that can be utilized to differentiate the genotypes between parents. Based on this result, ID96 race and 10 markers were used for screening and developing a near-isogenic line of *Pita-2* with Ciherang background. © 2019 Friends Science Publishers

Keywords: *Pita-2*; Ciherang; IRBLta2-Re; ID96

Introduction

Blast is one of prevalent diseases in paddy plants, which are caused by *Pyricularia grisea* fungus. Blast disease in a plant can be recognized easily by naked eye, and it can be observed from the leaves, neck and panicle branch of the paddy plant that have brown-yellow spots. The spots for sensitive varieties will extend in humid conditions and become larger until its margin cannot be seen clearly. The spots are surrounded by pale yellow color and called "halo area" (Ou, 1979; Yuliani and Maryana, 2014).

Usually, paddy plants are not resistant to blast disease;integrated control is used for blast prevention after anticipating this disease (Santika and Sunaryo, 2008). In an effort to control thisdisease, growing blast-resistant paddyis a strategy that shouldbe adopted (Toha, 2007). The formation of blast-resistant superior varieties begins with a screening of strains having blast resistance genes. Thesestrains will be used as a donor for the development of the blast resistance variety and expected to be a donor parent for further development of backcrossing population, for obtaining blast-resistant lines (NIL=near-isogenic lines) and generating multiline varieties (Suwarno and Soenarjo, 2001). Moreover, blast monogenic strain can be used to test the pathogenicity of the blast races.

The interaction of pathogenswhich have the avirulent gene (*avr*) to the resistant plant with the R gene will induce the defense system from the plant so that the plant can avoid pathogen infection (Ou, 1985). There are two types of blast resistance,partial resistance (PR) and complete resistance (CR). CR is controlled by major genes and are race-specific.This resistance is caused by incompatible interactions between the host cell and the pathogenic race; this inhibits the development of pathogens and is generally controlled byone gene. On the contrary, PR occurs through compatible interactions, thus inhibitingthe development of pathogens; this resistance reaction is controlled by many genes that usually against no specificraces (Koizumi, 2006). One of the blast monogenic strains that carry the blast resistance genes is IRBLta2-Re, which possesses blast

To cite this paper: Fitriah, N., U. Widyastuti, Suharsono, S. Nugroho, Suwarno and Miftahuddin, 201x. Screening of markers and blast isolates for blast resistance selection on backcross-population from crossing Ciherang and IRBLta2-Re. *Intl. J. Agric. Biol*., 22: 468–474

resistance genes called *Pita-2*. This is gene obtained from the Reiho parent, japonica paddy (Tsunematsu *et al*., 2000; Fukuta *et al*., 2009). IRBLta2-Re has a broader spectrum of resistance than IRBLta-CP1 tested with 196 local Chinese blast isolates (Lei, 2014). The IRBLta-CP1 monogenic strain also carried *Pita-2* gene, that was obtained from the Pi. 4 parents, and there was no difference of virulence frequency value between IRBLta2-Re and IRBLta-CP1 monogenic strains. The *Pita-2* gene of IRBLta-CP1 has a CR (Koizumi, 2006; Lei, 2014). The *Pita-2* gene is located on chromosome 12; it is close to the *Pita* gene (Rybka *et al*., 1997) The *Pita* gene is located near the centromere (Hayashi *et al*., 2006).

The improvement in Ciherang characteristic was made by introgressing of the *Pita-2* gene using *Marker Assisted Backcrossing Selection* approach (MABS). MABS has two methods, the phenotype and the genotype selection. The phenotype selection requires standard blast isolates, this isolate will be used to select blast resistance properties in backcross-population of Ciherang and IRBLta2-Re. The genotype selection exercised molecular markers to select *Pita-2* gene from donor parents in backcross-population of Ciherang and IRBLta2-Re.

The study was aimed to obtain standard blast isolates and molecular markers of the *Pita-2* gene. Subsequently, this result will be used to detect and select Ciherang that brings blast resistance genes (*Pita-2* gene) in backcross-population.

Materials and Methods

Virulence Determination of Blast Isolates

The examination and determination of blast isolate were basedon the method suggested by Mogi *et al*. (1991). The seven Indonesian paddy varieties were Ciherang, IR64, Asahan, Limboto, Inpago 8, Inpari 23 and Inpara 9; two blasts monogenic varieties were IRBLta2-Re and IRBLkp-K60; Kencana Bali was usedas a susceptible control.10paddy plants were tested by the blast disease using eight blast races. The blast isolateswere ID36, ID96, 033, 073, 133, 173, dc4 and 001 races.

10 varieties of paddy were grown in a plastic tray sized 20 cm x 15 cm x 5 cm and repeated thrice. The paddy plant was maintained and fertilized with urea once a week and placed in a greenhouse. The plant was ready for blast inoculation after ± 21 days, according to Hayashi *et al*. (2009). After that, the observation scores of blast symptom was determined by looking at the blast attacks on the leaves, as well as the distribution and size of spots that appeared on leaves. The level of blast attack was based on IRRI Evaluation Standard System (2014). While, the disease index 0 indicated the criteria of being highly resistant, 1-3 corresponded to being resistant, 4-6 related to moderate resistance or moderate susceptibility and 7-9 characterized being susceptible. This observation can be observed in Table 1 and Fig. 1.

Molecular Markers Screening of the *Pita-2* Gene

The molecular markers for the blast resistance gene of the IRBLta2-Re are used (Koide *et al*. 2009) along with the primer designed from single nucleotide polymorphisms (SNP)data of IRBLta2-Re. The *Pita-2* markers are selected based on the position of the flanking markers or close to the *Pita-2* gene with a distance of 0.5 cM - 1.0 cM from the *Pita-2* gene. Molecular markers screening of *Pita-2* was performed on both crosses parents, Ciherang and IRBLta2-Re, with the aim to detect *Pita-2* gene introgression of donor parents. The primary test begins with DNA isolation from the parent's plants of IRBLta2-Re and Ciherang. DNA isolation was performed by the modified Fulton *et al*. (1995) method. DNA quality and quantity measurements were performed using the UV Nanophotometer Implen tool. The quality of DNA was determined based on the ratio value at an absorbance of 260-280 nm, and DNA purity with values ranging from 1.8 to 2.0 (Sambrook *et al*., 1989). The primer testing of *Pita-2* gene was conducted using PCR machine from AB Applied Biosystems 2720 Thermal Cycler, with total reaction volume of 10 μL, consisting of 5 μL Fermentas mastermix, 0.25 μL (5 μM) forward and reverse primer of *Pita-2*, actin for DNA control, 100 ng/μL DNA template and 3,4 μL ddH_2O. The amplification reaction program begins with pre-denaturation at 95°C for 4 min. It is then forwarded with 35 cycles consisting of a denaturation stage at 94°C for 1 min, primer annealing at 55-65°C for 1 min, and primary elongation at 72°C for 1 min. This reaction ended with one primary elongation reaction cycle at 72°C for 5 min. The PCR amplification results were checked by electrophoresis on 2% agarose gel (w/v). The electrophoresis was run in Tris-Acetate-EDTA (TAE) buffer 1x at 50 volts for 45 min. Subsequently, the agarose gel was stained with Ethidium bromide (EtBr) 1% for 15 min and rinsed with water for 5 min. The agarose gel was observed under UV light with a chemidoc gel system (Biorad) device.

Results

Testing and Standards Determination of Blast Isolates

The result from the determination of blast standard isolate obtained ID96 isolate. Further, this race will be used for blast resistance selection in the backcross population of Ciherang and IRBLta2-Re. The ID96 race has low virulence to the IRBLta2-Re monogenic strain and high virulence to Ciherang and Kencana Bali as the vulnerable control shown in Fig. 2.

Mostly Ciherang leaves demonstrateblast symptoms. The observation of blast spots on Ciherang paddy leaves displayed that Ciherang paddy had no resistance to blast disease. Similarly, results reported in Ciherang's testing in

Table 1: Field key to the visual assessment of leaf blast severity *Standard Evaluation System For Rice* (IRRI, 2014)

Scale	Description	Diseased leaf area (%)
0	No typical susceptible lesion observed	0
1	Rapid observation does not reveal leaf lessions, but careful scrutiny of each row reveals few lesions	<0.3
2	Rapid detection detects a few lesions	0.3-0.9
3	Several lesions are randomly scattered within a plot and the lesion number on an infected leaf range from 1 to 4	1-2
4	Upper leaves are uniformly dotted with blast lesions but without necrotic (brown) leaf tips. A few to several leaves are brown	3-7
5	Several to many lower leaves become necrotic and few dead leaves are observed. Tips of several upper leaves show brown color and begin to fold	8-14
6	Lower leaves are uniformly exhibiting brown color and several dead leaves are visible. Tip necrosis of upper leaves is predominant	15-24
7	Tips of most upper leaves are curling. Middle and lower leaves are brown. Several plants or tiller are stunted/dead	25-39
8	Extensive leaf curling and browning of upper and middle leaves are prevalent. Plants generally stunted and many plants are dead	40-65
9	Majority of plants are severely stunted, brown and dead. Only a few to several plants have green leaves with heavy infection	>65

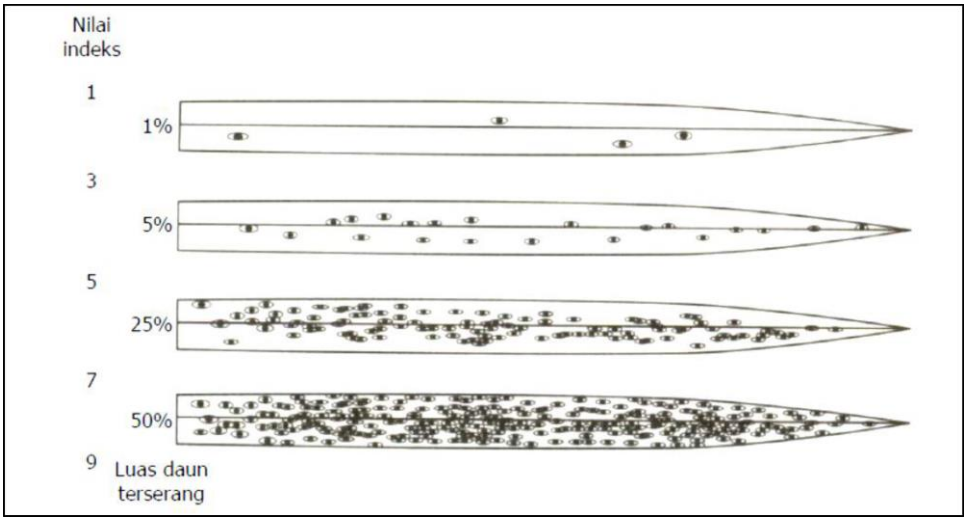

Fig. 1: The index value and corresponding levels of severity for leaf spot disease (*e.g.*, brown spot, blast) "Standard Evaluation System For Rice" (IRRI, 2014)

an endemic area without the artificial inoculation showed a high disease intensity of 55.60% (Suganda *et al*., 2016). This result proves that Ciherang is one of the paddy varieties that are susceptible to blast disease. Blast spots on Ciherang grow significantly and lead to some plants' death. The size of the spots enlarged after an incubation period of 14 days and formed halo area on the leaves with a scale of 5-7. The intensity blast leaves on blast standard testing is shown in Table 2.

The data obtained from testing of eight blast races on Ciherangrevealed that the highest blast intensity can be found inrace isolate ID96, 073 and 133. These results reinforced the hypothesis that Ciherang paddy has no resistance to blast disease specifically in races ID96 and 133, where most of tested Ciherang plants died after the incubation of two weeks. The highest blast-leaf intensity was obtained on Kencana Bali paddy, which was used as a susceptible control. Most of the blast-leaf symptomswere observed for the first-time on Kencana Bali paddy compared to other paddy plants with various attacks scores depending on blast races. Kencana Bali is an upland paddy, one of the differential paddies in Indonesia that are used as a susceptible control on blast race test (patotype test). Kencana Bali is susceptible to all *P. oryza* pathogens thatexist in Indonesia. The use of control plants also checks whether the inoculum that is used for blast testing has good performance.

Table 2. illustrates the value of blast intensity. The data indicatethat all varieties have different reactions to the eight blast races tested, ranging from resistant, moderate; and susceptible. The reaction of Ciherang paddy for all blast races was susceptible, but none of the races were susceptible for dc4 and 001 isolate races. These races were not virulent (low virulence) on Ciherang or other varieties.

The performance of eight blast races on blast monogenic strain IRBLta2-Re and IRBLKp-K60 that carry *Pita-2* and *Pik* gene showed very low leaf-blast intensity value (Table 2). The leaf-blast intensity percentage from

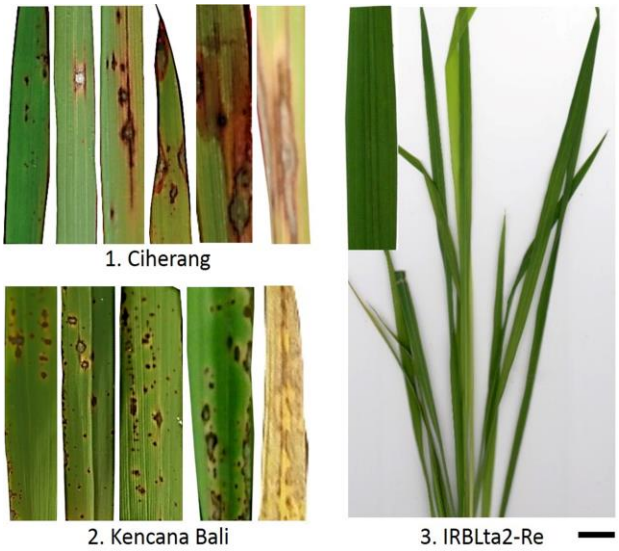

Fig. 2: Symptoms blast spots on paddy leaves. **1**. Ciherang paddy showed varying reactions to all blast races tested; the spots on leaves varied in size and number. After the incubation period and two times observation at seventh and 14th days, the obtained blast-leaf score shifted from around 0-5 to 3-7 for the race ID96, ID36, 133, 073, 033, 173, and dc4, where the blast spot developed rapidly. **2**. Kencana Bali as susceptible control have a blast spotting, which varied the obtained result blast score around 0-7, depending on blast race with two times observation at seventh and 14th days; the spot develops rapidly for all blast races tested and several plants have died. **3**. The IRBLta2-Re monogenic strain showed the resistant reaction in all blast races tested on a scale of 0-1; the incubation period of seven days followed by observation after 14 days showed that the spots were not developed

the monogenic strain was 0-15.6% for IRBLta2-Re and 1.1-19.2% for IRBLKp-K60 and all isolates. These results represent that IRBLta2-Re has abroad-spectrum of blast resistance. The same results were obtained from spectrum testing of IRBLta2-Re and IRBLta-CP1 with 196 local Chinese blast isolates. It was found that resistance spectra of IRBLta2-Re monogenic strains are broader than IRBLta-CP1 (Chen *et al*., 2014). These results were reinforced, with the outcome of blast standard testing on the five selected monogenic strains and Situ Paten gang with 14 blast isolates showing that the IRBLta2-Re monogenic strain has high resistance to blast (Suwarno *et al*., 2014).

The Molecular Markers Screening of *Pita-2* Gene

Table 3 presents the results of the molecular markers screening of the *Pita-2* gene to IRBLta2-Re and Ciherang paddy. The data of marker screening of the *Pita-2* gene on both parents showed from 23 markers used in this research in Koide *et al*. (2009), only 10 of them can detect the *Pita-2* gene in IRBLta2-Re as donor parent. The 10 markers were grouped into two types, the dominant and the codominant markers. The codominant marker is a marker that appears

Table 2: The intensity of blast leaves on the ten rice varieties in standard blast isolates.

No	Varieties	Intensity of blast leaves (%)							
		1	2	3	4	5	6	7	8
1	IR64	5.7	25.2	16.7	6.9	12.2	4.4	0.7	6.7
2	Ciherang	9.3	15.9	14.1	3.7	37	13.7	2.2	1.2
3	IRBL kp-K60	9.3	11.1	19.2	1.1	1.9	1.2	2.6	1.9
4	IRBLta2-Re	7.1	12.5	15.6	0	3	5.3	0.5	0
5	Kencana Bali	70.9	77.8	61.1	9.9	8.9	56.8	0.7	0
6	Asahan	8.5	11.5	15.6	4.1	7.8	8.1	1.5	2
7	Limboto	29.6	33.7	38.9	6.7	43.7	34.8	1.7	1.6
8	Inpago 8	10.4	26.7	29.3	8.9	24.1	11.1	4.1	1.2
9	Inpari 23	11.9	22.2	22.6	16.7	52.6	7.4	3.3	2.2
10	Inpara 9	4.4	5.2	12.6	7.4	32.6	8.1	1.5	0.9

1 = race 033; 2 = race 073; 3 = race 133; 4 = race 173; 5 = race ID96; 6 = race ID36; 7 = race dc4; 8 = race 001

Table 3: The screening of the *Pita-2* gene markers from the IRBLta2-Re and Ciherang

No	Markers name	PCR	PCR product	Annotation
1	ta642-1F	A	306	Dominant; IRBLta2-Re, *Pita-2*
2	ta801-1F	A	306	Dominant; IRBLta2-Re, *Pita-2*
3	ta577 -1F	A	303	Dominant; IRBLta2-Re, *Pita-2*
4	RM7102	B	300	Dominant, IRBLta2-Re, *Pita-2*
5	RM28009	E	300	Dominant; IRBLta2-Re, *Pita-2*
6	RM247	B	200-210	Codominant, *Pita-2*
7	RM27946	A	173-350	Codominant, *Pita-2*
8	ta3-1F	A	173-300	Codominant, *Pita-2*
9	ta3-2F	A	173-300	Codominant, *Pita-2*
10	RM101	B	300-324	Codominant, *Pita-2*
11	SNP-IRBLta2-Re-12-1	C	350	Dominant; IRBLta2-Re
12	SNP-IRBLta2-Re-12-2-1	D	369	Dominant; IRBLta2-Re
13	SNP-IRBLta2-Re-12-3-1	D	332	Dominant; IRBLta2-Re
14	SNP-IRBLta2-Re-12-4	C	328	Dominant, IRBLta2-Re
15	SNP-IRBLta2-Re-12-5	D	320	Dominant, IRBLta2-Re

A = Amplification Reaction began with pre-denaturation temperature of 94°C = 4 minutes; then continued with 35 cycles consisting of temperature denaturation of 94°C = 1 minute; annealing 55°C = 1 minute, elongation of primary temperature of 72°C = 2 minutes; and ended with one primary elongation reaction cycle at 72°C for 7 minutes
B = Amplification reaction equal to A but with primer (annealing) temperature 60°C = 1 minutes
C = Amplification reaction is equal to A but with annealing temperature 60°C = 45 minutes, 30 cycles
D = Amplification reaction equal to A but with primer (annealing) temperature 65°C = 45 minutes, 30 cycles
E = Amplification reaction equal to A but with primer (annealing) temperature 69°C = 45 minutes, 28 cycles

in both the parents and has different band sizes; these markers are used as recombination marker to identify generations from the parents, whereas the dominant marker is a marker that appears in only one of the parents, whichcan be Ciherang or IRBLta2-Re. In this study, the desired marker is a marker that can detect *Pita-2* gene introgression of the donor parent in the backcross populations;thus the selected markers appear only in IRBLta2-Re as the donor parent. Eight markers candidates were obtained that will used in genotype selection on backcross-populationfrom the marker screening in both parents (Table 3).

In this experimentation, the markers that designed from the SNP data of IRBLta2-Re that located on chromosome 12. The primary design was made using Web SNAPER at https://pga.mgh.harvard.edu/cgi-

bin/snap3/websnaper3.cgi. The primers were selected with the consideration of the following items; the last four base similar to SNP data of IRBLta2-Re, TM temperature in the range of 60-65°C, and the GC content around 50-60%. Seven SNP primers were tested on both parents, and five primers were derived that only appeared on IRBLta2-Re.

Discussion

The symptom of blast fungus was seen on the seventh day, or sooner than that for susceptible varieties. The blast attack rate was highly dependent on environmental conditions, paddy varieties, and blast races. The success of plant infection by blast fungi began in the early stages. This stage was very crucial as it was in this stage that the cycle infection began the three-celled conidia, which started to stick hydroponically on the cuticle surface of the leaf and developed into a narrow germ tube; after that, it flattened and got fixated on the leaf surface, differentiating into appressorium. Appressorium underwent a melanized process; it produced a large turgor and formed a penetration peg that has a tapered base part, which pierced the cuticle and then enteredinto the epidermis,forming hyphae. The hyphae invasively invaded the plasma membrane and attacked the epidermal cells. Lesions occurred between 72-96 h after infection and sporulation arisen under damp conditions (Wilson and Talbot, 2009). The ability of blast fungus to infect plants tested in an early stage was affected by several techniques. First, the temperature should not be high at the time of infection; there are two choices of ideal infection time; the first is around 4-6 pm, and the Sec. if the weather is cloudy in the morning, it can be inoculated around 7-8 am. Sec., the condition of the inoculation chamber should be wet and moist. Third, before being infected by the blast, the plants must be watered 1-2 h previous to inoculation. Last, the inoculum source should be fresh and have a density of inoculum (suspension of conidia) above 3×10^5 spores/mL.

The value of leaf-blast intensity obtained in this study varied from scale 0 (no symptoms) to scale seven. The blast symptoms were grouped into three classes; the resistant group scored between 0-1, the score three formed the moderate group; and the susceptible group showed between 5-7 score. The resistant group's reaction represented an incompatible interaction of plant and pathogen, where the interaction between the *R* genes in resistant plants and the *avirulent* (*avr*) genes on blast pathogens cause a hypersensitive response in the plant and activate the plant defense system. This interaction of R gene and the avirulent gene were based on the gene-for-gene hypothesis by Flor, and this theory evidenced in the blast pathosystems (Silue *et al*., 1992). The reactions data of IRBLta2-Re to all blast races tested are the incompatible reaction shows in Table 2. This result reinforced with another research that the avirulence value and virulence of the *Pita-2* genes from Pi No.4 and Reiho are 84 and 16%, respectively. The IRBLta2-Re resistance included complete resistance. Moderate reaction showed a compatible plant-pathogeninteraction that was affected by other genes possessed by differential paddy (Kobayshi *et al*., 2006).

The interaction between susceptible plants and pathogens can cause the disease called a compatible reaction. Ciherang reaction to the tested eight blast races are shown in Table 2. The score frequency varied depending on the blast races.

Blast resistance genes have been cloned; so far seven genes have been cloned. All resistance genes have sequences NBS (nucleotide binding sites) and LRR (leucine-rich repeat) domain. The products of the NBS and the LRR domains will interact with the avirulent (*avr*) gene from pathogens, where the resistance type is one gene to one gene (Koide *et al*., 2009). The *avr* gene product for the *Pita* gene is an avr-pita that binds specifically the LRR domain of Pita protein *in vitro* binding assay onto two yeast hybrid system. These results suggested that the product of resistance genes directly binds to the effector gene product of the pathogen and induces the defense system (Jia *et al*., 2000).

The screening result of the gene marker of the *Pita-2* obtained markers that can be used to detect the presence of the *Pita-2* gene (Table3). These markers referred to the work of Koide *et al*. (2009) who have summarized the location of blast resistance genesin paddy and produced a genetic map with the previously reported gene positions. Moreover, the five primers designed based on SNP IRBLta2-Re data, have been done successfully for the amplification within the IRBLta2-Re. The SNP markers combined with markers that have been referredby Koide *et al*. (2009) were the combination that applied to the genotype selection in the backcross populations between Ciherang and IRBLta2-Re.

The markers designed according to the DNA polymorphism between the resistant and the susceptible varieties; this difference is located within or around the blast resistance genes, these markers can indirectly select phenotype and should have a strong association and be closely linked to the gene that encodes the phenotypes; markers unrelated to the genes can minimize.

The selection of markers was based on the genetic distances between markers and target genes. The genetic distances of markers should have a distance 1 cM from target genes, using at least three pairs of markers flanking the gene.It is expected that these markers can detect the target genes from the donor. In general, the associations become stronger when tightly linked markers are used; however, markers that are closely related to the gene do not work when recombination occurs during the cross-process using MAS method (Fjellstorm *et al*., 2004). As a result, a plant thatdoes not have a target gene can be selected as false positives. So, to reduce the possible false positive selection, a confirmation was required to the introgression of the genetic region where the target gene was used as a

specific gene marker or two strongly linked markers were used on both sides of the gene region (Koide *et al*. 2009).

There are two type of markers in this study; SSR (simple sequence repeats) marker and SNP (single nucleotide polymorphisms) marker. The SSR marker became one of the most widely molecular markers used in plant breeding. The advantages of these markers are the fact that they can be applied to a small amount of DNA; thus they can fit for small samples, for example, for the genotyping analysis. The selection can happen at the seedling stage, so it saves costs and is profitable for the breeding program (Koide *et al*., 2009).

Currently, SNP marker is the most widely developed marker. This marker can distinguish one base of an organism such as point mutation. The development of SNP-based markers has performed, for example, in the system that used specific PCR primers from nine blast resistance genes that were obtained from SNP and Indel information. The genotype of the SNP can be easily analyzed with a presence or absence of PCR amplification products (Hayashi *et al*., 2006). The high numbers and the widespread of SNPs and Indels in the paddy genome can produce specific markers corresponding to the genomic target region. SNP technological advances are helpful and facilitate the work in the field of breeding especially plant breeding.

Conclusion

The results showed that ID96 race has low virulence on IRBLta2-Re monogenic line and high virulent on Ciherang and Kencana Bali. Therefore, the ID96 race is selected as blast isolate for blast resistance selection. The molecular analysis using 30 markers showed 10 markers that can discriminate between parents genotypes. Based on this result, ID96 race and 10 markers were used for screening to develop a near-isogenic line for *Pita-2* with Ciherang background.

Acknowledgments

We would thank the U.S. government that has supported and funded this research through USAID-Indonesia Smart Agri-Coalition for Indonesia program as well as Kebun Percobaan Muara of Indonesian Center for Rice Research, Laboratory Biorin, PPSHB, IPB, Laboratorium Biotechnology of Indonesia Institute of Sciences and Mrs. Dra. Anggia Nasution for her help and Mrs. Dr. Ir. Utut Widyastuti MSi for all her knowledge and guidance in this research.

References

Chen S., J. Su, J. Han, W. Wang, C. Wang, J. Yang, L. Zeng, X. Wang, X. Zhu, C. Yang, 2014. Resistance spectrum assay and fine mapping of the blast resistance gene from a rice experimental line, IRBLta2-Re. *Euphytica*, 195: 209–216

Fjellstorm, R., C.A. Conaway-Bormans, A.M. McClung, M.A. Marchetti, A.R. Shank and W.D. Park, 2004. Development of DNA markers suitable for marker assisted selection of three *Pi* genes conferring resistance to multiple *Pyricularia grisea* pathotypes. *Crop Sci*., 44: 1790–1798

Fukuta, Y., D. Xu, N. Kobayashi, M. Jeanie, T. Yanoria, A. Hairmansis and N. Hayashi, 2009. Genetic characterization of universal differential varieties for blast resistance developed under the IRRI-Japan Collaborative Research Project unsing DNA markers in rice (*Oryza sativa* L.). *In: Development and Characterization of Blast Resistance Using Differential Varieties in Rice*, pp: 35-68. Fukuta, Y., C.M.V. Cruz and N. Kabayashi (Eds.). Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Fulton, T.M., J. Chunzoongse and S.D. Tanksley, 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Mol. Biol. Rep*., 13: 207–209

Hayashi, K., H. Yoshida and I. Ashikawa, 2006. Development of PCR-based allele-specific and in Del marker sets for nine rice blast resistance genes. *Theor. Appl. Genet*., 113: 251–260

Hayashi, N., N. Kobayashi, C.M. VeraCruz and Y. Fukata, 2009. Protocol for the sampling of disease specimens and evaluation of blast disease in rice. *In: Development and Characterization of Blast Resistance Using Differential Varieties in Rice*, pp: 17–33. Fukuta, Y., C.M. Vera Cruz and N. Kabayashi (Eds.). Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

IRRI, 2014. *Standard Evaluation System for Rice*, 5th edition, pp: 1–55. The International Rice Research Institute, Los Baños, Philippines

Jia, Y., S.A. McAdams, G.T. Bryan, H.P. Hershey and B. Valent, 2000. Directinteraction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO J*., 19: 4004–4014

Kobayshi, N., M.J.T. Yanoria and Y. Fukata, 2006. Differential varieties bred at IRRI and virulence analysis of blast isolates from the Philippines. *In: A Differential System for Blast Resistance for Stable Rice Production Environment*, pp: 17–30. Fukuta, Y., C.M.V. Cruz and N. Kabayashi (Eds.). Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Koide, Y., N. Kobayashi, D. Xu and Y. Fukuta, 2009. Review: resistance genes and selection DNA markers for blast disease in rice (*Oryza sativa* L.). *Jpn. Agric. Res. Quart.*, 43: 255–280

Koizumi, S., 2006. Durability of resistance to rice blast disease. *In: A Differential System for Blast Resistance for Stable Rice Production Environment*, pp: 1–10. Fukuta, Y., C.M. Vera Cruz and N. Kabayashi (Eds.). Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Lei, C., 2014. Potential of rice blast management and mechanism exploration by using monogenic line in Yunnan Province, China. *In: Annual Meeting for Blast Research Network for Stable Rice Production under a JIRCAS Research Procject in Bangkok.* October 27, 2014. Thailand

Mogi, S., B. Sugandi, Edwina and R. Cahyadi, 1991. *Establishment of the differential Variety Series for Pathogenic Race Identification of Rice Blast Fungus and the Distribution of Race Based on the new Differential Indonesia.* Rice Disease Study Group, Karawang, Jatisari, Indonesia

Ou, S.H., 1985. *Rice Disease*, 2nd edition, pp: 1–380. Common wealth Mycological Institute, Surrey, England

Ou, S.H., 1979. A Handbook of Rice Diseases: *In: Tropics*, pp: 1–76. International Rice Research Institute, Los Baños, Philippines

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. *Molecular Cloning: a Laboratory Manual*, 2nd edition, pp: 1–1546. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NewYork

Santika, A. and Sunaryo, 2008. The technique of testing of upland paddy strain against blast disease (*Pyricularia grisea*). *Bull. Tekn. Pert.*, l13: 5–10

Silue, D., J.L. Notteghem and D. Tharreau, 1992. Evidence of a gene-for-gene relationship in the *Oryza sativa-Magnaporthe grisea* pathosystem. *Phytopathology*, 82: 577–580

Suganda, T., E. Yulia, F. Widiantini and Hersanti, 2016. Disease intensity of blast disease (*Pyricularia oryzae* Cav.) of Ciherang rice variety at the endemic location and its effect on yield loss. *Agric. J.*, 27: 154–159

Suwarno, D.W., Utami, A. Hairmansis, A. Nasution, Santosa and A. Dadang, 2014. Genetic dan pathological studies for blast disease and preparation of multiline introduction in Indonesian. *In: Annual Meeting for Blast Research Network for Stable Rice Production under a JIRCAS Research Procject in Bangkok.* October 27, 2014. Thailand

Suwarno and E. Soenarjo, 2001. Breeding of upland rice in Indonesia. *In: Upland Rice Research in Indonesia,Current Status and Future Direction*, pp: 1–6. Kardin, M.K., I. Prasadja and M. Syam (Eds). Central Research Institute for Food Crops, Indonesia

Toha H.M., 2007. Increasing upland rice yield through varietal component on the application of ICM. *J. Pengkajian dan Pengembangan Teknologi Pertanian*, 26: 180-187

Tsunematsu, H., M.J.T. Yanoria, L.A. Ebron, N. Hayashi, I. Ando, H. Kato, T. Imbe and G.S. Kush, 2000. Development of monogenic lines of rice for blast resistance. *Breed. Sci.*, 50: 229–234

Wilson, R.A. and N.J. Talbot, 2009. Review: under pressure investigating the biology of plant infection by *Magnaporthe oryzae*. *Nat. Rev.*,7: 185-195

Yuliani D., Y.E. Maryana, 2014. Blast Disease Control Technology Integration on Rice in Sub-Optimal Land. *In: Proceedings of the National Seminar of Sub-optimal Land in Palembang,* Indonesia. September 26-27, 2014.

[Received 12 Mar 2018; Accepted 24 Dec 2018; Published (online) 12 Jul 2019]