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SCAR (Sequence Characterized Amplified Region) Analysis for Blast Resistant Evaluation on 12 Genotypes of Rice

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ABSTRACT

Resistance evaluation to blast disease (Pyricularia grisea) on 12 paddy genotypes was carried out in the green house by using spray inoculated method with race 033 and 041 of P. grisea , and SCAR (Sequence Characterized Amplified Region) marker by using Pib primer pairs. The results revealed that among 12 paddy genotypes were classified into six resistance groups. The first group comprised two genotypes (Jatiluhur and Asahan) having three resistance genes. The second group comprised two genotypes (Oryza malampuzhaensis and O. punctata) having two resistance genes against race 033 and 041. The third group had one resistance gene against race 033, comprised one genotype (Way Rarem). The fourth group comprised one genotype (Danau Tempe) having two resistance genes against 041 race and Pib. The fifth group comprised three genotypes (Kalimutu, Maninjau and Laut Tawar) having two resistance genes against race 033 and Pib. The sixth group comprised two genotypes (Kencana Bali and Cirata) having no resistance gene to blast race 033 and 041, and Pib. These results indicated that Pib gene did not confer resistance to race 033 and 041 of Pyricularia grisea. Resistance to race 033 and 041 might be controlled by different resistant gene.

Key words : SCAR, Blast resistant, Rice

INTRODUCTION

Rice blast, caused by the fungal pathogen *Pyricularia grisea*, is the most serious disease for upland. However, recently it has been reported that the pathogen also infest irrigated rice (Amir *et al.*, 2000). The fungus attacks leaves during early growth stages, develops lesions that are followed by premature leaf senescence of infected tissues, especially in case of heavy infections. After heading, the pathogen infects the panicles or the neck, giving high lost of yield. The use of resistant cultivars is the most effective means on controlling the diseases, however, the useful life span of many cultivars is only few years, due to breakdown of the resistance in the face of high pathogen variability of the fungus (Kiyosawa, 1982).

The genes conferring resistance to rice blast has been studied extensively. So far at least 30 resistance loci have been identified in rice (Inukai *et al.*, 1994), and several loci have recently been mapped by using Restriction Fragments Length Polymorphism (RFLP) markers (Yu *et al.*, 1996; Nakamura *et al.*, 1997). Wang *et al.* (1999) was successfully isolated and characterized *Pib* gene, one of the genes conferring resistance to rice blast disease, by using map-based cloning strategy. The availability of information regarding the complete sequence of *Pib* gene leads to the possibility of developing specific primers to mark the *Pi-b* gene. These markers are classified as Sequence Characterized Amplification Region (SCAR) markers, which offer advantage on accuracy over RAPD markers, since the primer consist of more than 20 bases, and simplicity over RFLP markers. Detection of SCAR markers does not need laborious steps of blotting, hybridization and detection (Sobir, 2000).

Resistance to blast diseases in rice is conferred by R-genes that named as *Pi* genes (Ou, 1985). The *Pi* genes act as major gene, which recognize specific rice blast race, following gene-for-gene hypothesis (Ebron *et al.*, 2002). To date 25 *Pi* genes have been identified already (Fukuta *et al.*, 2002), located in several loci on rice genome (Wang *et al.*,1999). To date, based on reactions pattern to 7 differential varieties, in Indonesia have been identified 27 races of *P. grisea* (Amir *et al.*, 2000), but was not available information, wheather resistance to each of these races controlled by specific

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Pi gene or not. The dominant *Pi-b* gene is confers high resistance to most Japanese blast race, but in Indonesia, is not well identified yet, particularly in what fungus race that the gene to be confers and what varieties that carrying the gene.

MATERIALS AND METHODS

Plant and Inoculum Materials

Eleven rice genotypes, consisting of two wild species of (1) *Oryza malampuzhaensis,* (2) *Oryza punctata* and nine cultivated varieties of (3) Jatiluhur, (4) Cirata, (5) Way Rarem, (6) Laut Tawar, (7) Maninjau, (8) Danau Tempe, (9) Kalimutu, (10) Asahan (as control of resistant genotype) and (11) Kencana Bali (as control of susceptible genotype) were examined.

Two races of fungus, race 033 and 041, were used in this experiment, since both of them widely found in paddy field in Indonesia (Amir *et al.*, 2000). Inoculation materials were developed from fresh isolated conidia from the leaf, which infected by *P. grisea* race 033 and 041. They were cultured in PDA (*Potato Dextrose Agar*) media for 5 days, subsequently transferred to OMA (*Oat Meal Agar*) media and cultured for 10 days.

Blast Infection Assays

All evaluated genotypes were planted in a culture box containing clay soil 6 days after germination in greenhouse. Inoculation was conducted to the rice leaf 18 days after planting, by using compressor connected glass atomizer; each box was sprayed with 50 ml fungus spore, containing $3x10^6$ spore/l. After inoculation the plants were placed in humid room for 2x24 hours, and then transferred into greenhouse for observation.

Observation of diseases infection intensity was conducted 5 and 9 days after inoculation based on IRRI criteria, and obtained data were analyzed by the following equation (IRRI, 1996).

$$Z = \Sigma \frac{nivi}{NV}$$

Where: Z = infection intensity
ni= plant number-i
vi= score of plant number-i
N= number of observed plant
V= maximum score base on IRRI criteria

SCAR Analysis

DNA sample of the 12 evaluated genotypes were extracted from 1 g young leaves of 6 days rice seedling by using CTAB extraction method (Doyle and Doyle, 1987) with slight modification (Sobir, 2000). Quantity and quality of extracted DNA was examined by electrophoresis method.

SCAR analysis was conducted by amplification DNA samples of 12 genotypes of rice by using pair of 20 mer *Pib* primer designed from mRNA sequence of *Pib* gene (Wang *et al.*, 1999). The primers sequence are 5'-AGGGAAAAAT GGAAATGTGC-3' (sense) and 5'-AG TAACCTTCTGTGCCCAA-3' (anti-sense). Polymerase Chain Reaction (PCR) was performed in 25 ml reaction containing of 2.5µL of 10X buffer, 1.5µL of 25 mM $MgCl_2$, 1µL of 2.5 mM dNTPs, 1µL of 10 pM of each primers, 1µL of 100 ng DNA template and 1 unit of Taq DNA polymerase enzyme. Amplification was carried out by using Perkin Elmer 9700 PCR machine under following conditions Pre-PCR at 94^{0}C for 5 minutes, followed by 30 cycles of 94^{0}C for 30 seconds, 58^{0}C for 30 seconds and 72^{0}C for 1 minute. PCR was stopped at 72^{0}C for 7 minutes. PCR products were resolved in 1.0% of agarose gel.

RESULT

Observation was conducted on the susceptible type spot, as indicated by gray color on the center of the spot, since this spot is the source of conidia for secondary infection. Based on spot observation at 5 days after inoculation (dai) and 9 dai, infection intensity data were presented in Table 1. Infection intensity score below 25 indicates that the genotype is resistant and higher than 25 indicates that the genotype is susceptible. Based on the infection intensity score criteria, it was found that Danau Tempe, Cirata and Kencana Bali genotypes were susceptible to *Pyricularia grisea* race 033, and Laut Tawar, Maninjau, Kalimutu, Cirata and Kencana Bali genotypes were susceptible to *Pyricularia grisea* race 041.

Table 1. Infection intensity on 5 days after inoculation (dai) and 9 dai of the fungus race of 033 and 041 on 11 rice genotypes.

Genotype	Race 033		Race 041	
	5 dai	9 dai	5 dai	9 dai
Oryza malampuzhaensis	2.5	0.0	1.6	0.6
Oryza punctata	12.3	0.0	4.9	0.9
Jatiluhur	10.2	6.70	10.8	14.2
Asahan	0.0	0.0	3.2	4.0
Laut Tawar	22.7	20.2	30.3	38.0
Maninjau	11.7	10.3	43.0	43.6
Kalimutu	27.2	20.9	39.9	29.0
Way Rarem	3.7	10.0	27.7	24.0
Danau Tempe	18.5	33.9	11.3	9.9
Cirata	31.1	31.5	26.9	33.2
Kencana Bali	90.5	58.6	76.0	78.0

dai (day after inoculation)

Examination of *Pib* existence in the genome of evaluated genotypes was detected with single band of 730 base pairs of amplification product *Pib* SCAR primer. The *Pib* SCAR marker analysis revealed that Jatiluhur, Maninjau, Kalimutu, Way Rarem, Danau Tempe and Cirata genotypes carried *Pib* gene (Figure 1). Correlation analysis showed that the existence of *Pib* gene is not corresponding to the resistance responses of the evaluated genotypes, neither to race 033 or race 041.

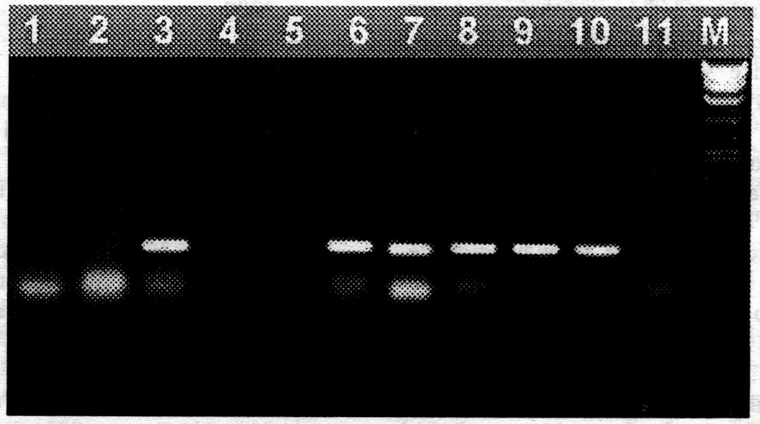

Figure 1. SCAR marker analysis by using *Pib* primer pair. Lane no 1 to no 11 represent *Oryza malampuzhaensis*, *Oryza punctata*, Jatiluhur, Asahan, Laut Tawar, Maninjau, Kalimutu, Way Rarem, Danau Tempe, Cirata, and Kencana Bali, respectively. M is DNA size markers (λ–DNA/*Hind*-III digest).

Based on resistance responses to race 033 and race 041, and existence of Pib gene in the genome, the evaluated genotypes can be classified into six groups. First group consisted of Jatiluhur and Asahan, which were resistant to race 033 and race 041, and carried *Pib* gene. Second group consisted of *Oryza malampuzhaensis* and *Oryza punctata*, which were resistant to race 033 and race 041, but did not carry *Pib* gene. Third group consisted of Kalimutu, Maninjau and Laut Tawar, which were resistant to race 033 and carried *Pib* gene. Fourth group consisted of Danau Tempe, which were resistant to race 041 and carrying *Pib* gene. Fifth group consisted of Way Rarem, which was resistant to race 033 only. Sixth group consisted of Cirata and Kencana Bali, which were susceptible to race 033 and 041, did not carry *Pib* gene (Table 2).

Table 2. Classifications of rice genotypes base on resistance respond to P. grisea race 033 and race 041, and existence *Pib* gene in the genome

Group	Genotype	Resistance		
		Race-033	Race-041	*Pib*-SCAR
I	Jatiluhur	+	+	+
	Asahan	+	+	+
II	*Oryza malampuzhaensis*	+	+	0
	Oryza punchtata	+	+	0
IIII	Kalimutu	+	0	+
	Maninjau	+	0	+
	Laut Tawar	+	0	+
IV	Danau Tempe	0	+	+
V	Way Rarem	+	0	0
VI	Cirata	0	0	0
	Kencana Bali	0	0	0

DISCUSSION

To date 25 *Pi* genes have been identified (Fukuta *et al.*, 2002), located in several loci on rice genome (Wang *et al.*, 1999). The *Pi* genes act as major gene, which recognize specific rice blast race, following gene-for-gene hypothesis to induce defense mechanism again blast fungus (Ebron *et al.*, 2002). Therefore differences of resistance among rice varieties to different races might due to different type of *Pi* genes that carried by certain rice varieties. Subsequently, differences of resistance response among rice genotypes to *P. grisea race* 033 and 044 (Table 2), was probably due to resistance to race 033 conferred by different *Pi* gene that confers resistance to race 041. In other hand, since the existence of *Pib* gene was not associated to resistance response to both races (Table 2), this indicated that *Pib* gene did not confer resistance to *P. grisea* race 033 and 044.

SCAR marker analysis result showed that the *Pib* gene did not exists in the genome of *Oryza malampuzhaensis* and *Oryza punctata,* indicating that *Pib* gene was not originated from both wild species. According to Ebron *et al.* (2002), *Pib* gene is already carried by several *Oryza sativa* var. indica genotypes such as Indonesian indigenous Peta variety. In other experiment we found that *Pib* gene is carried by wild species *Oryza rufipogon* (unpublished data), thus raise possibility that *Pib* gene is originated from *Oryza rufipogon.*

Wide range availability of *Pi* genes in rice gene pool in conferring resistance to several races of blast fungus *Pyricularia grisea* (Ebron *et al.*, 2002) raise the possibility to develop rice varieties with field resistance through pyramiding of several *Pib* gene into one genotype, as has been exhibited by Jatiluhur and Asahan.

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