Bul. Agron. (33) (1) 1 – 5 (2005)

SCAR (Sequence Characterized Amplified Region) Analysis for *Pi-b* and *Pi-ta* genes on 28 Genotypes of Rice

Sobir[1*], Santoso[2], Hajrial Aswidinnoor[1] and Mukelar Amir[2]

Received 16 February 2005/Accepted 11 April 2005

ABSTRACT

Evaluation to blast disease (Pyricularia grisea) resistance was carried out by using two SCAR (Sequence Characterized Amplified Region) markers of Pi-b and Pi-ta blast resistance genes, and spray-inoculation method with 10 races of P. grisea on 28 paddy genotypes, that consisted six wild genotypes of rice. The results revealed that among 28 paddy genotypes, fifteen genotypes carrying both genes including Oryza rufipogon; six genotypes carrying Pi-b genes including O. alta, two genotypes carrying Pi-ta gene, and five genotypes did not possess both gene including O. glumaepatula, O. officinalis, O. latifolia, and O. malapuzhaensis. Based on infection intensity, the evaluated genotypes were vary in responses to different ten races of P. grisea, indicated that the evaluated genotypes were vary in carrying Pi genes. Analysis in detail indicated that existence of Pi-ta gene associated with lower infection intensity caused by P. grisea race 063 C.

Keywords: SCAR markers, rice blast resistance, Pyricularia grisea

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 of them have recently been mapped by using Restriction Fragments Length Polymorphism (RFLP) markers (Yu *et al*., 1996; Nakamura *et al*., 1997). Wang *et al.* (1999) has successfully isolated and characterized *Pi-b* 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 Pib 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 (Lee, 1995).

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 seven differential varieties, 27 races of *P. grisea* have been identified in Indonesia (Amir, *et al*., 2000), but there is not available information, whether the resistance to each of these races controlled by specific *Pi* gene or not. The dominant *Pi-b* gene confers high resistance to most Japanese blast race. However, in Indonesia, this gene has not been identified yet particularly which fungus race this gene conferred to and which varieties carrying the gene. Mc Couch *et al*. (1994) indicated that *Pi-ta* gene located at chromosome 9 or 12.

In order to evaluate the existence of *Pi-b* and *Pi-ta* genes in cultivated rice in Indonesia along with wild species of rice, molecular analysis by utilizing *Pi*-b and *Pi-ta* SCAR (Sequence Characterized Amplified Region) markers, and spray-inoculation method were conducted to 22 cultivated rice and 6 wild species.

[1] Department of Agronomy, Faculty of Agricultur, Bogor Agricultural University, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680
Telp./Fax. (0251) 629353 (*Author for correspondence)
[2] Rice Research Institute, Bogor

MATERIALS AND METHODS

Plant and inoculum materials

Twenty eight rice genotypes, consist of six wild species of (1) *Oryza alta*, (2) *Oryza glumaepatula*, (3) *Oryza officinalis*, (4) *Oryza rufipogon*, (5) *Oryza latifolia*, (6) *Oryza malampuzhaensis* and twenty two cultivated varieties of (7) Sutrapendek, (8) Kalimutu, (9) Cisanggarung, (10) Gajah Mungkur, (11) Krueng Aceh, (12) Simariti, (13) Cisokan, (14) Sirendah Putih, (15) Sagi, (16) Cisadane, (17) Klemas, (18) Seratus Malam, (19) Bonti, (20) IR-64, (21) Grogol, (22) Asahan, (23) Cabacu, (24) Salumpikit, (25), Bulan Sabit Putih (26) Sepulo, (27) Moroberekan and (28) Kencana Bali as susceptible control genotype.

Ten races of fungus collection of Rice Research Center, (1) race 001 C, (2) race 003 C, (3) race 063 C, (4) race 103 C, (5) race 113 C, (6) race 201 C, (7) race 023 SK, (8) race 133 SK, (9) race 153 L and (10) race 173 L were used in this experiment. Inoculums were prepared from fresh infected leaves by those of *P. grisea* races. Subsequently they were cultured in PDA (Potato Dextrose Agar) media for 7 days at 25^0C, transferred to OMA (Oat Meal Agar) media and incubated for 10 days at 25^0C.

SCAR analysis

Two pairs of SCAR primers designed from mRNA sequence of *Pib* gene (Wang *et al*., 1999) and mRNA sequence of *Pi-ta* (Bryan *et al*., 2000). The SCAR primers sequences for Pib gene is AgggAAAAATggAAATgTgc and AgTAAccTTcTgcTgcccAA; and for *Pi-ta* is gTcAggTTgAAgATgcATAgc and cAATgccgAgTgTgcAAAgg.

DNA sample of the 28 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. Quantity and quality of extracted DNA was examined by electrophoresis method.

SCAR analysis was conducted by amplification total DNA samples of 28 genotypes of rice by those SCAR primers. Polymerase Chain Reaction (PCR) was performed in 25 ml reaction consist of 2.5μL of 10X buffer, 1.5μL of 25 mM MgCl2, 1μL of 2.5 mM dNTPs, 1μL of 10 pM of each primers, 1μL of 100 ng DNA template and 2 unit of *Taq*-DNA polymerase enzyme (Takara). Amplification was carry out by using Perkin Elmer 9700 PCR machine under following conditions Pre-PCR at 94^0C for 5 minutes, followed by 30 cycles of 94^0C for 30 seconds, 58^0C for 30 seconds and 72^0C for 1 minute. Stop PCR at 72^0C for 7 minutes. PCR products were resolved in 1.0% of agarose gel.

Blast infection assays

All evaluated genotypes were planted in a culture box containing clay soil 6 days after germination in greenhouse. Inoculation was performed to the rice leaf 18 days after planting (having 3-4 leaves), by using compressor connected glass atomizer; each box was sprayed with 50 ml fungus spore, containing of $3x10^5$ spore/ml. Following inoculation, the plants were placed in moist chamber for 2x24 hours, and then transferred into greenhouse for resistance evaluation. Observation of diseases infection intensity was conducted at 7 days after inoculation based on IRRI scoring standard (IRRI, 1996), and grouped as follow; score 0-2 = resistant (R), score 3= moderate resistant (MR), score 4-6 = moderate susceptible (MS) and score 7-9 = susceptible (MS)

RESULTS

Examination of *Pib* existence in the genome of evaluated genotypes was detected about 500 bp amplification product of *Pi-b* SCAR primer (Figure 1A), and 2 genotypes out of 6 wild rice genotypes found carrying *Pi-b* specific fragment. Moreover, from 22 cultivated rice genotypes, 19 genotypes carrying *Pi-b* specific fragment, indicated they were possessed *Pi-b* gene (Table 1).

Table 1. Existence of *Pi-b* and *Pi-ta* genes in 28 evaluated genotypes and their resistance responses to 10 races of

Pyricularia grisea.

No	Genotypes	*Pib*	*Pi-ta*[1]	001 C	003 C	063 C	103 C	113 C	201 C	023 SK	133 SK	153 L	173 L
1	*Oryza alta*	1	0	R	MR	S	MR	MR	MR	R	MS	S	S
2	*O. glumaepatula*	0	0	R	R	S	R	S	R	R	S	MR	S
3	*O. officinalis*	0	0	R	S	S	MR	R	MR	MR	S	R	R
4	*O. rufipogon*	1	1	MR	S	MR	MR	S	S	S	S	S	R
5	*O. latifolia*	0	0	MR	R	S	MR	S	MR	S	S	S	MR
6	*O. malampuzhaensis*	0	0	MR	R	S	R	R	R	S	S	MR	S
7	Sutrapendek	0	1	R	MR	MR	R	MR	R	R	S	S	S
8	Kalimutu	1	0	R	R	MR	MR	S	R	MR	MR	S	S
9	Cisanggarung	1	1	R	S	S	S	S	MR	S	S	S	S
10	Gajah Mungkur	1	1	MR	MR	MR	R	MR	R	R	MS	MR	S
11	Krueng Aceh	1	1	MR	MR	S	R	MR	R	S	S	MR	S
12	Simariti	1	1	R	R	R	MR	S	MR	MR	S	S	S
13	Cisokan	1	1	R	MR	MR	S	S	MR	MR	S	S	S
14	Sirendah Putih	1	1	MR	R	MR	R	S	R	S	MR	MS	S
15	Sagi	1	0	R	R	R	MR	MR	R	R	MS	MS	S
16	Cisadane	1	1	R	R	MR	MR	S	MR	MR	S	S	S
17	Klemas	1	1	R	MR	MR	MR	MR	S	MR	S	MR	S
18	Seratus Malam	0	1	MR	R	MR	R	S	R	R	MS	MR	S
19	Bonti	1	1	R	R	R	R	MR	S	R	MR	MR	S
20	IR-64	1	1	MR	MR	S	MR	R	MR	R	MR	MS	S
21	Grogol,	1	1	R	R	R	R	S	S	R	MR	MR	MS
22	Asahan	1	1	R	R	R	R	MR	MS	R	MR	MR	MR
23	Cabacu	1	0	R	R	R	R	MR	MR	R	MR	S	MR
24	Salumpikit	1	1	MR	R	R	R	MR	MR	R	MS	S	MS
25	Bulan Sabit Putih	1	0	R	R	R	R	MS	R	MR	S	S	S
26	Sepulo	1	0	R	R	R	R	S	MR	R	MR	MR	MS
27	Moroberekan.	1	1	R	R	R	R	MR	MR	R	R	MR	S
28	Kencana Bali	0	0	S	S	S	S	S	S	S	S	S	S

Notes: 1: amplify DNA fragment from genomic DNA using SCAR primers; 0: not amplify
R: resistant; MR: moderate resistant; MS: moderate susceptible; S: susceptible

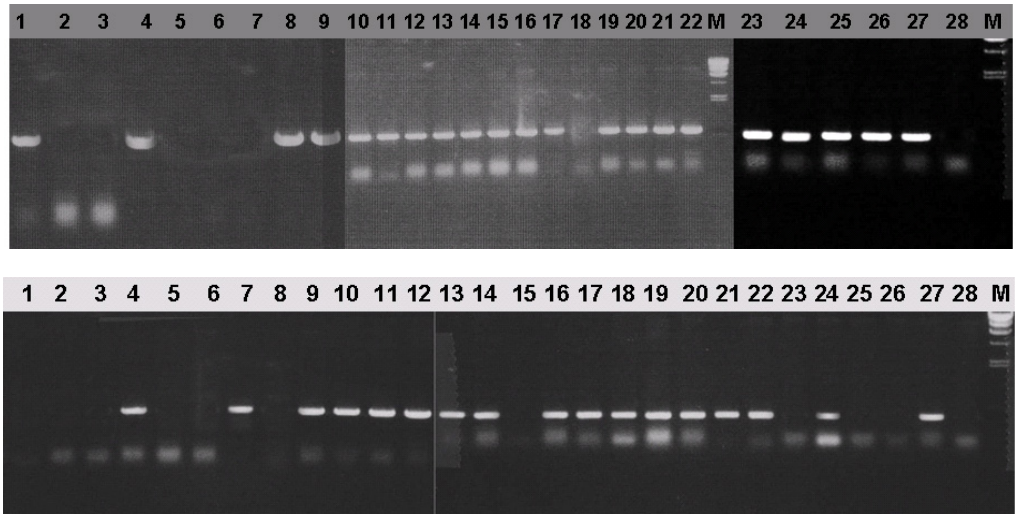

Figure 1. Amplification result of *Pi-b* primer pair (A) and *Pi-ta* primer pair (B). Lanes 1-28; PCR products from DNAs of *Oryza alta, O. glumaepatula, O. officinalis, O. rufipogon, O. latifolia, O. malampuzhaensis,* Sutrapendek, Kalimutu, Cisanggarung, Gajah Mungkur, Krueng Aceh, Simariti, Cisokan, Sirendah Putih, Sagi, Cisadane, Klemas, Seratus Malam, Bonti, IR-64, Grogol, Asahan, Cabacu, Salumpikit, Bulan Sabit Putih, Sepulo, Moroberekan and Kencana Bali respectively. M; DNA size markers (λDNA/*Hind*III digest).

Primer pair of *Pi-ta* was amplified DNA fragment about 550 bp (Figure 1B), and two genotypes out of six

wild rice genotypes found carrying *Pi-ta* specific fragment. Furthermore, from 22 cultivated rice genotypes, 16 genotypes carrying *Pi-ta* specific fragment, indicated they are having *Pi-ta* gene (Table 1).

Analysis on wild species of rice indicated that *Oryza rufipogon* carrying of both *Pi-b* and *Pi-ta, O. alta* carrying *Pi-b,* however *O. glumaepatula, O. officinalis, O. latifolia,* and *O. malampuzhaensis* are not having both genes. Furthermore, on cultivated genotypes, 14 genotypes carry both genes, five genotypes carrying *Pi-b*, two genotypes carrying *Pi-ta*, and one genotype Kencana Bali as susceptible control not having both gene.

Resistance analysis of rice genotypes to ten races of *P. grisea* was observed base on the susceptible type spot at 7 days after inoculation (dai), as indicated by gray color on the center of the spot, since this spot is the source of conidia for secondary infection. Based on their infection intensity data the rice genotypes categorized into R: resistant; MR: moderate resistant; MS: moderate susceptible; S: susceptible to each races (Table 1). The Blast infection assays results revealed that each evaluated rice genotypes were vary in responses to different races of blast fungus. However, Kencana Bali as susceptible control was showed susceptible response to all *P. grisea* races indicated that inoculums in this experiment were virulent.

DISCUSSION

Differences of each evaluated rice genotypes response to different races of *P. grisea* in blast infection assay, lead to suggestion of differences in genetics properties corresponding to blast disease resistance of evaluated genotypes. Mc Couch *et al*. (1994) reported that to date at least 30 *Pi* genes have been identified in rice, that located in several loci on rice genome (Wang *et al*., 1999; Fukuta *et al*., 2002), and recognize specific rice blast race (Ebron *et al*., 2002) following gene-for-gene hypothesis to induce defense mechanism again blast fungus (Flor, 1971;). Therefore, differences of resistance among rice genotypes to different races might due to different type of *Pi* genes that carried by each rice genotypes. In practical use, the resistant and moderate resistant genotypes were possible to utilize them as alternative varieties in endemic blast diseases area, as well as gene resources for breeding purpose.

SCAR marker analysis result showed that most cultivated genotypes are having *Pi-b* and *Pi-ta*, indicated that both genes has been already carried in rice breeding program from their parental genotype. According to Ebron *et al*. (2002), *Pi-b* gene is already carried by several *Oryza sativa* var. indica genotypes such as Indonesian indigenous Peta variety. 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) was raise possibility to develop rice varieties with field resistance through pyramiding of several *Pi* genes into one genotype, as has been exhibited by 14 rice genotypes. Moreover, Naqvi and Chatoo (1996) reported Moroberekan carrying *Pi-10* gene.

In order to elucidate association of existence *Pi-b* and *Pi-ta* genes with resistance response to specific *P. grisea* races, correlation analysis was performed. The analysis result indicated that *Pi-b* gene not associated with infection intensity of any evaluated *P. grisea* races, however, *Pi-ta* gene associated with infection intensity of race 063 C at correlation coefficient of -0.55, indicated that the existence *Pi-ta* gene related to lower infection intensity of race 063 C. However, on Cisanggarung, Krueng Aceh and IR-64 existence of *Pi-ta* not associated with resistance to race 063 C. Those exceptional results probably due to *Pi-ta* gene should be associated with existence *Pi-ta2* gene to confer resistance to *P. grisea* (Bryan *et al*., 2001). The association of *Pi-ta* gene with another genes to confer resistance to *P. grisea* is resemble to those of *RPP* and *RPS* genes family in Arabidopsis (Glazebrook, 2001), and *Mla* gene in Barley (Wei *et al*., 2002).

REFERENCES

Amir, M., A. Nasution, Santoso. 2000. Inventarisasi ras *P. grisea* di daerah Sukabumi, Jawa-Barat, musim tanam 1995-1998. Prosiding Kongres Nasional Perhimpunan Fitofatologi Indonesia, Purwokerto. 2000.

Bryan, G. T., K. S. Wu, L. Farral, Y. Jia, H. P. Hersey, S. A. McAdams, K. N. Faulk, G. K. Donaldson, R. Tarchini, B. Valent. 2000. tA single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell. 12:2033-2045

Doyle, J.J., J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 12:13-15.

Ebron, L.A., Y. Fukuta, T. Imbe, H. Kato, H. Tsunematsu, M.J.T. Yanoria, G.S. Kush, M. Yokoo. 2002. Identification of blast resistance genes in IRRI rice varieties. Proceeding of Japan Breeding Society Annual Meeting. Obihiro, August 26-27, 2002.

Flor, H.H. 1971. Status of the gene-for-gene concept. Annu. Rev. Phytopathol. 28:275-296.

Fukuta, Y., E. Araki, J.M. Yanoria, L.A. Ebron, D. Mercado-Escueta, H. Tsunematsu, H. Kato, T. Imbe, G.S. Kush. 2002. Development of

differential varieties and near-isogenic lines to rice blast resistance gene at the International Rice Research Institute. Proceeding of Japan Breeding Society Annual Meeting. Obihiro, August 26-27, 2002.

Glazebrook, J. 2001. Genes controlling expression of defense responses in *Arabidopsis* — 2001 status. Curr. Opin. Plant Biol. 4:301–308.

Inukai, T., R.J. Nelson, R.S. Zeigler, S. Sarkarung, D.J. Mackill, J.M. Bonman, I. Takamura, T. Konoshita. 1994. Allelism of blast resistance genes in near-isogenic lines of rice. Phytopthol. 84: 1278-1283.

IRRI. 1996. Standard evaluation system for rice (fourth edition). IRRI. Philippines. 52 p.

Kiyosawa, S. 1982. Genetic and epidemiological modeling of breakdown of plant disease resistance. Annu. Rev. Phytopathol. 20:93-117.

Lee, M. 1995. DNA markers and plant breeding programs. Adv. Agron. 55:265-344.

Mc Couch, S.R., R.J. Nelson, J. Tohme, R.S. Ziegler. 1994. Mapping of blast resistance genes in rice. p. 167-186. *In:* Ziegler R.S., S.A. Leong, P.S. Teng (eds) Rice blast disease. CAB International. Manila. Philiphines.

Nakamura, S., S. Asakawa, N. Ohmido, K.Fukui, N.Shimizu, S. Kawasaki. 1997. Construction of an 800-kb contig in the near-centromeric region of rice blast resistance gene *Pi-ta* using a highly representative rice BAC library. Mol. Gen. Genet. 254: 611-620.

Naqvi, N. I., B. B. Chattoo. 1996. Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice. Genome. 39: 26-30.

Ou, S.H. 1985. Rice Disease. 2nd ed. Commonwealth Mycological Institute, Kew, UK. p: 109-183.

Wang, Z.X., M.Yano, U. Yamanouchi, M. Iwamoto, L. Monna, H. Hayasoka, Y. Katayose, T. Sasaki. 1999. The *Pi-b* gene for rice blast resistance belongs to the nucleotide binding and leucine rich repeat class of plant disease resistance genes. Plant J. 19: 55-64.

Wei, F., R.A. Wing, R.P. Wise. 2002. Genome Dynamics and Evolution of the *Mla* (Powdery Mildew) Resistance Locus in Barley. Plant Cell. 14:1903–1917.

Yu, Z.H., D.J. Mackill, J.M. Bowman, S.R. McCouch, E. Guiderdoni, J.L. Notteghem, S.D. Tanksley. 1966. Molecular mapping of genes for resistance to rice blast (*Pyricularia grisea* Sacc.). Theor. Appl. Genet. 95:859-863.