



Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*)

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ABSTRACT. Among 120 simple sequence repeat (SSR) markers, 23 polymorphic markers were used to identify the segregation ratio in 320 individuals of an F₂ rice population derived from Pongsu Seribu 2, a resistant variety, and Mahsuri, a susceptible rice cultivar. For phenotypic study, the most virulent blast (*Magnaporthe oryzae*) pathotype, P7.2, was used in screening of F₂ population in order to understand the inheritance of blast resistance as well as linkage with SSR markers. Only 11 markers showed a good fit to the expected segregation ratio (1:2:1) for the single gene model (d.f. = 1.0, P < 0.05) in chi-square (χ^2) analyses. In the phenotypic data analysis, the F₂ population segregated in a 3:1 (R:S) ratio for resistant and susceptible plants, respectively. Therefore, resistance to blast pathotype P7.2 in Pongsu Seribu 2 is most likely controlled by a single nuclear gene. The plants from F₂ lines that showed resistance to blast pathotype P7.2 were linked to six alleles

of SSR markers, RM168 (116 bp), RM8225 (221 bp), RM1233 (175 bp), RM6836 (240 bp), RM5961 (129 bp), and RM413 (79 bp). These diagnostic markers could be used in marker assisted selection programs to develop a durable blast resistant variety.

Key words: Rice blast (*Magnaporthe oryzae*); Disease resistance; Simple sequence repeat markers; F₂ population

INTRODUCTION

Rice blast, caused by the pathogenic fungus *Magnaporthe oryzae*, is one of the important rice diseases, causing great damage to rice yield because of its wide distribution and destructiveness under favorable conditions (Ou, 1980). Blast can be managed by providing multi-genetic resistance against a wide spectrum of blast races in rice cultivars (Correa-Victoria et al., 2002). This can be done by pyramiding the resistance genes into cultivar or rice varieties. Resistance to blast has been classified into complete and partial resistance (Wang et al., 1994). Complete resistance is a qualitative character and race specific controlled by a major gene. Meanwhile, partial resistance is a quantitative character and non-race specific, which is controlled by many genes known as quantitative resistance loci (Koizumi, 2007). However, if the resistance is highly partial, it can also be controlled by a major gene and is race specific. The use of molecular markers in many aspects of rice studies has been increasing considerably. Microsatellites or simple sequence repeats (SSRs) are widely used in rice genetic studies. SSRs are highly polymorphic genetic markers and because of their widespread distribution in the genome, high allelic diversity and inherent potential have become a valuable source of genetic markers. SSR markers have been applied to analyze diversity (Cho et al., 2000; Harrington, 2000) and to locate genes and quantitative trait loci (QTLs) on rice chromosomes (Zou et al., 2000; Bres-Patry et al., 2001; Moncada et al., 2001). Several microsatellite markers with publicly available databases closely linked with the *Pi-b*, *Pi-k^h* and *Pi-ta²* resistance genes have been identified (Fjellstrom et al., 2002). To date, more than 40 major blast resistance genes have been identified (Pan et al., 1999; Chauhan et al., 2002; Berruyer et al., 2003; Sallaud et al., 2003; Liu et al., 2004; Zhu et al., 2004). The presence of markers tightly linked to resistance genes will allow selection and maintenance of the desirable resistant genotypes in breeding process (Hittalmani et al., 1995; Naqvi and Chattoo, 1996). The objective of our study was to analyze SSR markers associated with rice blast resistance genes in an F₂ population derived from Pongsu Seribu 2 resistant variety and Mahsuri, a susceptible rice cultivar.

MATERIAL AND METHODS

Plant materials

An F₂ population derived from a cross between a local rice variety, Pongsu Seribu 2 (resistant variety), and a susceptible rice cultivar, Mahsuri (Figure 1), was genotyped with selected SSR markers associated with blast resistance genes. Pongsu Seribu 2 is a resistant variety that possesses broad-spectrum resistance to blast fungal isolates; it originate from the Malaysian Rice Research Centre, Malaysian Agricultural Research and Development Institute

(MARDI). Pongsu Seribu 2 was discovered to be resistant to many Malaysian blast pathotypes, similar to the famous donor resistant varieties, including Tadukan from the Philippines and Tetep from Vietnam (Jia et al., 2003). Mahsuri was obtained by crossing Taichung 65 and Mayang Ebos 80 in Malaysia (Yamakawa et al., 1977). Mahsuri has wide adaptability and good eating quality. Unfortunately, this cultivar is very susceptible to blast. Currently, it is used as a check variety for blast screening.



Figure 1. Rice cultivars Pongsu Seribu 2 (PS2) (resistant) and Mahsuri (Mh) (susceptible) inoculated with pathotype 7.2 of *Magnaporthe oryzae*. Severe blast lesions are observed in Mh and few lesions in PS2. The photo was taken 10 days after inoculation.

Genomic DNA isolation

Total genomic DNA was extracted from fresh frozen leaf tissues of 4-week-old individual F_2 and parental plants using the CTAB method, as described by Doyle and Doyle (1990) with some modifications and following the protocol described by McCouch et al. (1988). About 1 g leaf tissue was ground in liquid nitrogen and transferred to 15 mL pre-heated CTAB extraction buffer containing 100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA, pH 8.0, and supplemented with 30 μ L β -mercaptoethanol. Samples were incubated in a water bath at 60°C for 1 h by gently shaking the tubes at 5-min intervals. After incubation, an equal volume of chloroform-isoamyl alcohol (24:1, v/v) was added to the mixture, then the tube was gently inverted to homogenize the mixture at room temperature for 2 min prior to centrifugation. The samples were centrifuged at 5000 rpm for 10 min at 25°C using the Centrifuge 5810 (Eppendorf, Germany). The supernatant aqueous layer was transferred to a new 50-mL Falcon tube and 0.6 volume of cold (-20°C) isopropanol was added to each sample. The mixture was gently inverted and incubated on ice for 30 min. DNA was spooled out from the tube, transferred to a new micro-centrifuge tube and centrifuged at 12,000 rpm for 1 min. The DNA pellet of each sample was washed with 70% cold ethanol, followed by a quick centrifugation; the supernatant was gently removed and, after drying in air, the DNA was resuspended in 100 μ L TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). RNA was removed by adding 2 μ L (10 g/mL) RNase into the dissolved DNA of each sample and incubated for 30 min at 37°C. DNA was quantified by using nano-drop spectrophotometry (ND1000 Spectrophotometer). Re-quantification of DNA was performed on 1% agarose gel electrophoresis using 1X TAE buffer at 5-8 V/cm for 30 min and visualized under UV light by staining with 0.1 μ g/mL ethidium bromide and diluted with sterile distilled water to a concentration of 20 ng for polymerase chain reaction (PCR) analysis and kept in a freezer at -20°C.

Primers for SSR amplification

Primer sets of 120 microsatellites were used and selected from the Gramene database (www.gramene.org) related to blast resistance gene (*Pi-Genes*); it has been mapped by Wu and Tanksley (1993), Akagi et al. (1996), Temnykh et al. (2000), and McCouch et al. (2002). Primer pairs were optimized for PCR to amplify microsatellite loci. Parental varieties were used to identify SSR polymorphism associated with the rice blast resistance gene.

Polymerase chain reaction

PCR conditions for the SSR markers were as described in Panaud et al. (1996) and McCouch et al. (2002), with the following modifications: the total reaction was scaled down to 12.5 μ L: containing 40 ng template DNA, 100 μ M of each dNTP (0.2 mM each of dATP, dTTP, dGTP and dCTP), 1.5 mM $MgCl_2$, 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.0 μ M of each primer and 0.2 U *Taq* polymerase. PCR amplification was carried out in a thermocycler (PTC-200 Peltier Thermal Cycler DNA Engine) using the following temperature profile: initial denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min, followed by rapid cooling

to 4°C prior to analysis. For electrophoretic analysis, the PCR product was mixed with 2.5 µL 6X loading dye (0.2% bromophenol blue, 0.2% xylene cyanol dye and 30% glycerol in a Tris-EDTA buffer) and electrophoresis was run using a 3.0% agarose gel containing 0.1 µg ethidium bromide/µL in 1X TBE buffer (0.05 M Tris, 0.05 M boric acid, 1 mM EDTA, pH 8.0). The gel was run at a constant voltage of 90 V for 1 h and visualized under UV light, and analyzed using the Bio-Imaging System (Chemi-Genius, USA).

Genotyping for marker segregation

A total of 320 progenies were genotyped for SSR marker alleles. Alleles at the SSR loci were detected on 3% agarose gels. Alleles were scored based on the parental bands. A ladder was added with the first load, used to confirm the allele sizes observed in the parental survey. The plants that showed a pattern similar to the resistant parent alleles were scored as “R” and those with a banding pattern similar to the susceptible parent alleles, were scored as “r”, and the heterozygous plants were scored as “Rr”.

Disease evaluation in F₂ populations

To determine segregation patterns of blast resistance, F₂ seedlings were evaluated by inoculation with highly virulent pathotype P7.2 of *M. oryzae*. The blast isolate was obtained from the Rice Research Station, MARDI, Seberang Perai. Inoculation of blast pathogen was carried out as described by Chen (2001). Plants were grown in a green house at 25-30°C for 2 to 3 weeks, until they reached the four-leaf stage (Filippi and Prahbu, 2001). Twenty-day-old plants, with three or four fully expanded leaves, were inoculated by spraying with 25 mL aqueous spore suspension onto the leaves until run-off, using an atomizer connected to an air compressor. The blast conidial suspension was adjusted to 1 x 10⁵ per mL with sterilized deionized water. Inoculated plants were incubated in a moisture/dew chamber and the relative humidity (RH) was maintained at 100% for 24 h at 25° to 28°C, after which they were placed in the greenhouse (controlled environment) at temperatures ranging from 25° to 30°C (Filippi and Prahbu, 2001). The RH was maintained at above 90% by covering them with black netting while under moist jute sacks as well as watering them four to five times during the daytime. Disease assessment was scored 7 days after inoculation. Scoring was carried out based on the Standard Evaluation System (SES) of the International Rice Research Institute (IRRI, 1996) and the standardized protocol of Mackill and Bonman (1992). The blast lesion degrees (LD) were scored as follows: 0 = no evidence of infection; 1 = brown specking indicative of an RH response (<0.5 mm in diameter); 3 = brown lesions of 0.5-1.0 mm wide; 5 = round to elliptical lesions of 1-3 mm in diameter (or length) with a grey center; 7 = spindle shaped lesions with a grey center, and 9 = coalesced type 4 lesions across most of the leaf. For data analysis of the single-gene model, the rice plants showing lesion types 0 and 1 were considered to be resistant (R) and the plants showing lesions type 3 and above were considered to be susceptible (S) to the selected pathotype. A two-gene model was also analyzed by classification of resistance to pathotype P7.2 as R, moderately resistant (MR), moderately susceptible (MS), and S. The plants with lesion scores of 0 and 1 were considered to be R, 3 as MR, 5 as MS, and 7 and 9 as S (Bonman et al., 1989). According to Mendelian principle, the phenotypic segrega-

tion for a two-gene model is 9:3:3:1 for R:MR:MS:S, respectively. A test for epistasis was carried out by analyzing F_2 populations to determine whether they would segregate with a 15(R):1(S) ratio. The plants with disease reaction score of 0, 1, 3, and 5 were considered as R, while disease reaction scores of 7 and 9 were considered as S.

Statistical analysis

Segregation data were analyzed by the chi-square (χ^2) test. The chi-square analysis for the genotypic and phenotypic ratio was calculated by using the formula, $\chi^2 = (O - E)^2 / E$, where O is the observed value and E is the expected value. For the single-gene model and epistasis, each chi-square value was considered to be significant ($P \leq 0.05$) if its value was greater than 3.84, while for two independent genes if it was greater than 7.84.

RESULTS

SSR marker survey of parents and F_2 populations

Of 120 SSR markers, about 40% showed clear polymorphism between blast susceptible and resistant parents. Among these, the 23 best polymorphic markers were evaluated on 320 F_2 progenies derived from Pongsu Seribu 2 \times Mahsuri (Table 1). The patterns of all the markers varied in the segregating population. The banding patterns of microsatellite primer pairs for three polymorphic markers linked with blast Pi genes, RM168, RM8225, and RM1233, for 13 samples along with two parents are shown in Figures 2, 3 and 4, respectively.

Table 1. Information on the polymorphic microsatellite markers used for segregation analysis in rice.

SSR markers	Primer sequences (5'-3')		Chromosome	Repeat motif	Expected PCR product size (bp)
	F: sequence of forward primer	R: sequence of reverse primer			
RM1	GCGAAAACACAATGCAAAA	GCGTTGGTTGGACCTGAC	1	(GA) ₂₆	113
RM104	GGAAGAGGAGAGAAAGATGTGTTCG	TCAACAGACACACCGCCACCCG	1	(GA) ₈	222
RM428	AACAGATGGCATCGTCTTCC	CGCTGCATCCACTACTGTG	1	(AG) ₁₅	266
RM228	CTGGCCATTAGTCTTGG	GCTTGCGGCTCTGCTTAC	10	(CA) ₈ (GA) ₃₆	154
RM244	CCGACTGTTCGTCTTATCA	CTGCTCTCGGGTGAACGT	10	(CT) ₄ (CG) ₃ C(CT) ₆	163
RM304	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG	10	(GT) ₂ (AT) ₁₀ (GT) ₃₃	160
RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGTTGCGATAGAG	6	(GA) ₁₇	233
RM6836	TGTTGCATATGGTCTATTGA	GATACGGCTCTAGGCCAAA	6	(TCT) ₁₄	240
RM8225	ATGCGTGTTCAGAAATAGG	TTGTTGTATACCTCATCGACAG	6	A ₁₁ N(AAG) ₁₄	221
RM8226	TTAGGATACGGCTTCTAGGC	CGTAATTGTTGCATATGGTG	6	(AAG) ₁₄	251
RM340	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC	6	(CTT) ₇ T ₁ (CTT) ₁₄	163
RM206	CCCATGCGTTAACTAATTCT	CGTCCATCGATCCGATGAG	11	(CT) ₂₁	147
RM224	ATCGATCGATCTTACGAGG	TGCTATAAAAGGCATTCCGG	11	(AAG) ₈ (AG) ₁₃	157
RM229	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	11	(TC) ₁₁ (CT) ₃ C ₁ (CT) ₅	116
RM287	TTCCTGTTAAGAGAGAAATC	GTGTATTGGTGAAGAAC	11	(GA) ₂₁	118
RM332	GCGAAGGCGAAGGTGAAG	CATGAGTGTCTCACTCACCC	11	(CTT) ₅₋₁₂ (CTT) ₁₄	183
RM1233	GTGTAATCATGGCACGTG	AGATTGGCTCCTGAAGAAGG	11	(AG) ₁₅	175
RM5961	GTATGCTCCTCCTCACCTGC	ACATGCGAGTGTATGTAAC	11	(CAG) ₈	129
RM168	TGCTGCTTGCTGCTTCTTT	GAAACGAATCAATCCACGGC	3	T ₁₅ (GT) ₁₄	116
RM169	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTTATCCCTCC	5	(GA) ₁₂	167
RM413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	5	(AG) ₁₁	79
RM350	TGATCGTCGGATTCCCGGC	CCCCACCCTGCGCTTCTCC	8	(CT) ₁₀	208
RM1359	CTCGCAGGAAGAAGACAAC	CGCCGGCTGGTTAATTAATC	4	(AG) ₂₅	170

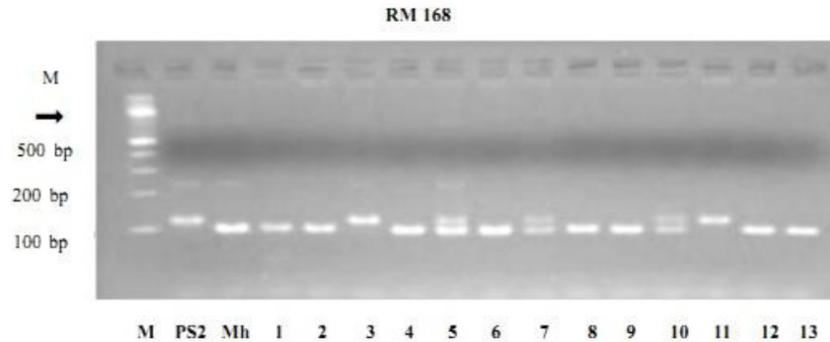


Figure 2. Marker banding patterns in an F₂ population of rice derived from Pongsu Seribu 2 (PS2) × Mahsuri (Mh) for SSR marker RM 168 linked to blast resistance genes. Running on 3% agarose gel stained with ethidium bromide only 13 samples plus the two parents (their corresponding parents) for each marker are shown. M = 100-bp ladder; PS2 = Pongsu Seribu 2; Mh = Mahsuri; lanes 1-13 = progenies.

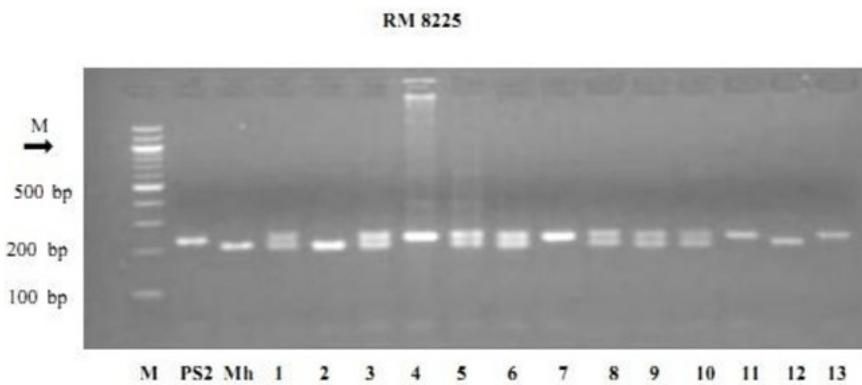


Figure 3. Marker banding patterns in an F₂ population of rice derived from Pongsu Seribu 2 (PS2) × Mahsuri (Mh) for SSR marker, RM 8225 linked to blast resistance genes. Running on 3% agarose gel stained with ethidium bromide only 13 samples plus the two parents (their corresponding parents) for each marker are shown. M = 100-bp ladder; PS2 = Pongsu Seribu 2; Mh = Mahsuri; lanes 1-13 = progenies.

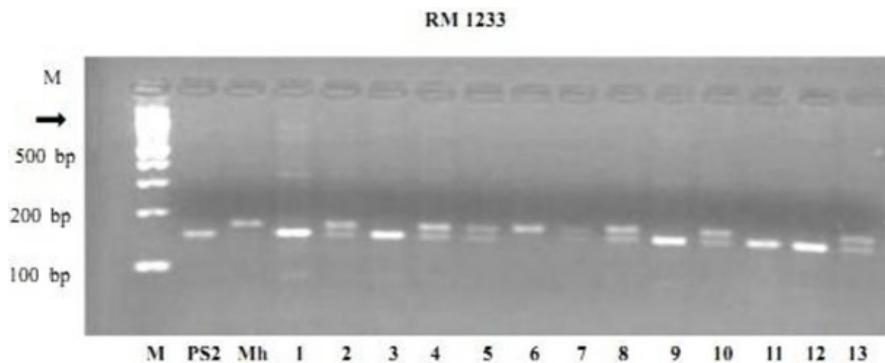


Figure 4. Marker banding patterns in an F₂ population of rice derived from Pongsu Seribu 2 (PS2) × Mahsuri (Mh) for SSR marker RM 1233 linked to blast resistance genes. Running on 3% agarose gel stained with ethidium bromide only 13 samples plus the two parents (their corresponding parents) for each marker are shown. M = 100-bp ladder; PS2 = Pongsu Seribu 2; Mh = Mahsuri; lanes 1-13 = progenies.

Marker segregation data analysis

Alleles at the SSR loci were scored based on the parental bands that were amplified as controls along with the F_2 individuals. The observed segregation ratio for resistance and susceptibility in F_2 lines for 23 polymorphic SSR markers is shown in Table 2. The chi-square (χ^2) analysis for 11 SSR markers, RM104, RM244, RM6836, RM8225, RM206, RM1233, RM5961, RM168, RM413, RM350, and RM1359, showed a good fit to the expected segregation ratio (1:2:1) for a single-gene model (d.f. = 1.0, $P < 0.05$). The rest of the markers did not fit the expected segregating Mendelian ratios.

Table 2. Marker analysis in F_2 lines derived from the cross between rice varieties Pongsu Seribu 2 \times Mahsuri.

Markers	Chromosome	Marker analyzed			χ^2 (1:2:1)	Probability
		RR = R	Rr = SG	rr = S		
RM1	1	86	138	96	6.68**	0.0354
RM104	1	90	150	80	1.88	0.3906
RM428	1	93	136	91	7.23**	0.0269
RM228	10	87	133	100	10.17**	0.0062
RM244	10	78	166	76	0.48	0.7866
RM304	6	66	160	94	4.9*	0.0863
RM527	6	106	144	70	11.3**	0.0035
RM6836	6	80	154	86	0.68	0.7118
RM8225	6	75	154	91	2.05	0.3588
RM8226	6	89	137	94	6.77**	0.0339
RM340	6	89	135	96	8.12**	0.0172
RM206	11	76	156	88	1.1	0.5769
RM224	11	104	144	72	9.6**	0.0082
RM229	11	68	159	93	3.92*	0.1409
RM287	11	98	136	86	8.1**	0.0174
RM332	11	104	159	57	13.82**	0.001
RM1233	11	86	147	87	2.12	0.3465
RM5961	11	86	146	88	2.48	0.2894
RM168	3	77	155	88	1.07	0.5857
RM169	5	92	135	93	7.82**	0.02
RM413	5	72	159	89	1.82	0.4025
RM350	8	73	163	84	0.87	0.6473
RM1359	4	88	153	79	1.12	0.5712

*0.05 significance level; **0.01 significance level. R = resistant; S = susceptible; SG = segregant; rr = plants with a banding pattern similar to the susceptible parent alleles; Rr = heterozygous plants. χ^2 = the actual value of the chi-square test for resistant/susceptible ratio.

Inheritance of blast resistance in an F_2 population

Inheritance studies of blast resistance against the most virulent local pathotype, P7.2 in F_2 progenies derived from the same cross, showed that resistance in this population segregated in a 3:1 ratio for plants with resistance and susceptibility (Table 3). The individuals of F_2 population showing alleles of RM168 (116 bp), RM8225 (221 bp), RM1233 (175 bp), RM6836 (240 bp), RM5961 (129 bp), and RM413 (79 bp) were resistant to pathotype P7.2.

Phenotypic segregation of the F_2 populations treated with pathotype P7.2 of *M. oryzae* for a two-gene model did not show a good fit to a 9:3:3:1 ratio ($\chi^2 = 43.92$, $P > 0.0001$). This indicates that resistance to blast caused by pathotype 7.2 in F_2 populations was most likely not controlled by two genes. The chi-square analysis of epistasis showed that the F_2 populations did not segregate in a 15R:1S ratio for resistant versus susceptible plants. Therefore, locus in-

teraction or epistasis of blast resistance did not occur in the F₂ population derived from Pongsu Seribu 2 and Mahsuri.

Table 3. Observed and expected segregation ratios of resistant and susceptible plants in the F₂ generation for the genetic cross between the rice cultivars Pongsu Seribu 2 × Mahsuri inoculated with pathotype 7.2 of *Magnaporthe oryzae*.

Generation	Pathogenicity assay			χ^2 (3:1)	P
	Reaction	Obs. No.	Exp. No.		
F ₂	Resistant (R)	227	240	0.70	0.1060
	Susceptible (S)	93	80	2.11	
	Total	320	320	2.81	

d.f. = 1.0; $\chi^2(0.05, 1) = 3.84$.

DISCUSSION

In segregation analysis, 11 polymorphic markers clearly showed goodness of fit to the expected segregation ratio for the single-gene model. The segregation ratio was not in agreement with the expected Mendelian ratio for 12 other polymorphic markers. Resistance in the F₂ population was inherited in a 3:1 ratio for plants with resistance versus susceptibility. Chi-square tests of data obtained from segregation of resistance of possible different gene models, i.e., two independent gene model and epistasis effect or two locus interactions, showed that blast resistance present in the F₂ population, specifically for pathotype P7.2, was not segregated into 9:3:3:1 or 15:1 ratios. We conclude that resistance to blast in Pongsu Seribu 2, specifically against pathotype P7.2, is mostly controlled by a single dominant gene. This situation is in agreement with the statement that the ability of a plant to express resistance is also dependent on the genotype of the pathogen. A rice plant cannot be resistant to an isolate of *M. oryzae* unless the pathogen has a gene that makes it virulent to the rice plant. An isolate of *M. oryzae* cannot be avirulent on the rice plant unless the rice plant has genes that make it resistant to that isolate (Ellingboe and Chao, 1994). Several studies have shown that partial resistance can be race specific (Bonman et al., 1989; Talukder et al., 2004; Lopez-Gerena, 2006). Our result is in agreement with a blast research done at IRRI in the Philippines, which indicated that one or two dominant genes present in the cultivars confer complete resistance against each fungal isolate (Yu et al., 1987). Meanwhile, Sharma et al. (2007) reported inheritance studies to determine genetic control of blast resistance in a cross involving the resistant (R) “Laxmi” cultivar and the susceptible (S) “Mahsuri” cultivar. The F₂ population segregated into 3R:1S, indicating that resistance in “Laxmi” was governed by a single dominant gene. This was also confirmed by the segregation patterns in the F₃ and F₄ generations. In another report, the resistance ratios of the subsequent F₂ progenies of Indian native rice cultivar Aus373 were governed by dominant alleles at two loci (Pan et al., 1999). Nevertheless, to understand the molecular mechanism of broad-spectrum resistance to rice blast and for further confirmation of the result reported here, additional genetics studies are needed to investigate whether qualitative and quantitative genes affect the level of the resistance in rice variety Pongsu Seribu 2.

The individuals of the F₂ population (derived from Pongsu Seribu 2 × Mahsuri) that had the alleles RM168 (116 bp), RM8225 (221 bp), RM1233 (175 bp), RM6836 (240 bp), RM5961 (129 bp), and RM413 (79 bp) were resistant to pathotype P7.2. The blast resistant plants had the alleles of these six SSR markers. This finding has potential for use in marker-assisted selection programs and confirmation of blast resistance genes to develop rice cultivars with durable blast resistance in Malaysian rice breeding programs.

CONCLUSIONS

Twenty-three polymorphic markers were used for the identification of segregation ratios in F_2 population. Chi-square analyses of 11 SSR markers showed an expected segregation ratio of 1:2:1, inherited in simple Mendelian fashion. Phenotypic data based on disease reaction of resistance and susceptibility to blast pathotype P7.2 also segregated in a 3:1 (R:S) ratio in the F_2 population. Therefore, resistance to blast pathotype P7.2 in Pongsu Seribu 2 is more likely controlled by a single nuclear gene. The plants resistant to blast pathotype P7.2 from F_2 lines had genotypes with SSR markers RM168, RM8225, RM1233, RM6836, RM5961, and RM413; these markers could be used for marker-assisted selection.

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