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MARKER ASSISTED SELECTION IN RICE BREEDING FOR BACTERIAL LEAF BLIGHT

Nguyen Thi Pha, Nguyen Thi Lang

ABSTRACT

One hundred and sixty six local accessions and 25 parent lines of hybrid rice were used as materials for screening leaf blight resistance using 10 international bacterial races in compared to check varieties. There were five local cultivars that resisted to bacterial races as IRBB21, three cultivars expressed resistant reaction as IRBB5 and 58 cultivars resisted to the race No 4 and No 6 as IRBB13. These cultivars were subsequent genotype analyzed using RG556, RG136 and PTA248 for detecting xa-5, xa-13 and Xa-21 genes. PCR products of materials using RG556 and PTA248 were failed to detect xa-5 and Xa-21. Marker RG136 was assisted to select five local rice accessions and three parent lines of hybrid rices containing xa-13 gene. A susceptible modern cultivar IR24 and resistant local rice Nang Som were used to develop backcross population to introgression of the recessive xa-13 gene. Bacterial blight resistant gene, xa-13, was not detected from the first BC generation. Screening 130 plants with the type of IR24 in BC_1F_2 population found 20 plants were resistant to at least 6 races including race 4 and race 6 (typical resistance phenotype of xa-13 gene). These plants were genotyped for 5 microsatellite markers (RM21, RM114, RM122, RM164, and RM190), of which 2 markers RM21 and RM190 showed polymorphism with the accuracy of 55% and 50%. In compare with BB pathogen reaction, these plants had not xa-13 gene but their resistance was affected by multiple genes at different loci.

Key words: Bacterial leaf blight, Marker-assisted selection (MAS), Polymerase chain reaction (PCR), STS marker

INTRODUCTION

Bacterial leaf blight (BB) caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*) is one of the major diseases infected rice (*Oryza sativa* L.) in the world. In some areas of Asia it can reduce crop yield by up to 50% (Khush et al. 1989) even up to 80% (Singh et al. 1977). The most effective approach to combat BB is using resistant varieties. There are 25 resistant genes have been reported to locate at various loci in different chromosomes of rice (Kinoshita 1995; Lin et al. 1998; Zhang et al. 1997). Many of them have been identified to link to RFLP, RAPD, microsatellite markers in genetic maps. The *Xa-2* was reported to link to Npb197 marker, located in chromosome 4 (Yoshimura et al. 1995). *Xa-4* gene was mapped on chromosome 11 and found to be closely linked to Npb78 marker with the distance of 1.7 cM (Yoshimura et al. 1995). McCouch et al. (1991) detected RG556 and RG207 linkage with the recessive *xa-5* gene. Five years later, McCouch et al. (1996) discovered two microsatellite markers, RM122 and RM13 linked to *xa-5* gene with the distance of 2.1 cM and 17.9 cM. The *xa-13* gene was found to be linked to RG136 marker with the distance of 3.8 cM in chromosome 8 (Zhang et al. 1996). The *Xa-21* gene was identified to link to RG103 (PTA248), RAPD248 and RAPD818 markers, located in the chromosome 11 (Ronald et al. 1992). Local rice varieties were often considerable to be a genetic source of disease resistance. Thanks to marker-assistance selection (MAS), some resistant genes have been detected in local rices from Mekong delta region such as: *xa-2* in Te Tep, *xa-5* in Ba Tuc, Giong Doi, Koi Bo Teng, *xa-13* in Ca Dung, Ba Tuc, Thom Lung, Ve Phich, Nep Hoa Vang, Nang Som (Nguyen Thi Lang and Bui Chi Buu 2002); or *Xa-21* in wild rice species as *O. longistaminata* (Khush et al. 1990). It will take you a long time with low efficiency in order to transfer these genes into modern rice varieties using conventional

breeding methods. Fortunately, some of these genes have been incorporated into modern rices using marker-assisted selection. For example, the *xa-5* gene has been transferred to IRBB5 from DZ192 variety with the assistance of RG556 marker (Yoshimura et al. 1995). The *xa-13* gene has been incorporated into IRBB13 variety from Long Grain variety using RG136 marker (Zhang et al. 1996). IRBB21 variety has been developed with the *Xa-21* gene from exotic germplasm of *O. longistaminata* using PTA248 marker (Ronald et al. 1992).

This study aims at detecting more major genes in local cultivars and assisting selection in rice breeding for leaf blight resistance using molecular marker.

MATERIALS AND METHODS

Plant materials

One hundred and sixty six local rice accessions were used for PCR polymorphism survey. These accessions were obtained from the genebank of Cuu Long Delta Rice Research Institute (CLRRI) which collected from 11 provinces of Mekong delta region. Twenty five parent lines of hybrid rice were also considered. Other BC generations of IR24/Nang Som were developed using IR24 as recurrent parent to change the recessive *xa-13* gene from Nang Som (donor) to BC populations. BC populations were used for PCR analysis after screening for BB resistance.

Target genes and DNA markers

The target genes selected for MAS experiment included two recessive genes, *xa-5* and *xa-13*, and a dominant one, *Xa-21*, conferring resistance to different BB races. These genes were detected using 3 STS markers to enrich BB resistance materials for rice breeding from local rices and hybrid rices. Three STS markers based on DNA sequences derived by manual sequencing from ends of RFLP clones RG556, RG136 and pTA248 (table 1). The Primer sequences for a PCR marker linked to *Xa-21* were from Chunwongse et al. (1993). The others were from Huang et al. (1997).

The MAS experiment for *xa-13* gene from BC populations was conducted to build the procedure of BB resistance in rice breeding. The STS marker designed from RG136 was the first alternative for MAS experiment. Five other microsatellite markers RM21, RM114, RM122, RM164, and RM190 were also used in this study to detect other genes may be incorporated in BC populations.

Table 1: STS markers and microsatellite markers used for PCR analysis

Marker STS marker	**Sequence**		**Chrm.**
RG556	F	TAGCTGCTGCCGTGCTGTGC	5
	R	AATATTTCAGTGTGCATCTC	
RG136	F	TCCCAGAAAGCTACTACAGC	8
	R	GCAGACTCCAGTTTGACTTC	
PTA248	F	AGACGCGAAGGGTGGTTCCCGA	11
	R	AGACGCGGTAATCGAAGATGAAA	
SSR marker			
RM21	F	ACAGTATTCCGTAGGCACGG	11
	R	GCTCCATGAGGGTGGTAGAG	
RM114	F	CAGGGACGAATCGTCGCCGGAG	3
	R	TTGGCCCCCTTGAGGTTGTCGG	
RM122	F	GAGTCGATGTAATGTCATCAGTGC	5
	R	GAAGGAGGTATCGCTTTGTTGGAC	
RM164	F	TCTTGCCCGTCACTGCAGATATCC	5
	R	GCAGCCCTAATGCTACAATTCTTC	
RM190	F	CTTTGTCTATCTCAAGACAC	6
	R	TTGCAGATGTTCTTCCTGATG	

DNA isolation

Protocol for DNA extraction was done according to the method suggested by Zheng et al. (1995). Healthy rice leaf sample (2cm long) was collected and placed in a labeled 1.5ml centrifuge tube on ice. Cut the leaf tissue into 0.5cm long segments and ground in a well of the thick polished glass rod with a small pestle after adding 400µl of extraction buffer (50mM tris-HCl pH 8.0, 25mM EDTA, 300 mM NaCl and 1% SDS). The tissue was ground until the buffer turns dark green. Added 400µl more of DNA extraction buffer and mixed in the well by pipetting. 400µl of the lysate was transferred to the original 1.5 ml of the leaf sample. Added 400µl chloroform and mixed well by inverting. Spin the tube for 30 sec in micro-centrifuge. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA precipitated using absolute ethanol. Spin for 3 min at 13.000 rpm and discarded the supernatant. After drying in air, the DNA was resuspended in 50µl of TE buffer (10mM tris-HCl pH 8.0, 1mM EDTA pH 8.0). DNA was done for PCR analysis. DNAs were stored at –20^{o}C for later use.

PCR amplification

PCR amplification was performed in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 to 1.0 unit of *Taq* polymerase, 200 µM each of dNTP, 0.25 µM of primer with 10 ng of genomic DNA per 20 µl using a thermalcycler. An initial denaturation was performed at 94^{o}C for 2 minutes prior to 30 cyles of denaturation at 94^{o}C (1 minute), annealing at 55^{o}C (1 minute), and extension at 72^{o}C (2 minutes). A final extension for 5 minutes at 72^{o}C will be performed. Polymorphisms in the PCR products will be detected after electrophoresis on 1.5% agarose gel for STS marker products and on 3% agarose gel for microsatellite before ethidium bromide staining.

Bacterial blight screening

All materials were grown in a CLRRI screen house. At the stage of 25-30 day old, the rice plants were inoculated with 10 international bacterial races of *Xoo* (PXO061, PXO086, PXO0340, PXO071, PXO0112, PXO099, PXO0145, PXO0280, PXO0339, PXO0341) using the clipping method (Kauffman et al. 1973) as compared to check varieties and parents of BC population. The inoculums were prepared by suspending the bacterial mass in sterile water to a concentration of # 10^9 cells per ml. Evaluation for resistance was conducted after 18 days inoculation by lesion length (LL) measurement. The distinction between resistant and susceptible plants was set at LL of # 5 cm. Plants with LL of < 5cm were scored as resistant and the others were susceptible.

RESULTS AND DISCUSSIONS

Detecting *Xa-21, xa-5* and *xa-13* genes in local rices and parent hybrid rices

Genomic DNAs of 166 local accessions and 25 parent lines of hybrid rice were used for PCR analysis using STS markers designed from pTA248, RG556 and RG136.

The first PCR marker by Chunwongse et al. (1993) linked to *Xa-21* did not detect any banding pattern of resistance as IRBB21 in all materials, and they did not contain *Xa-21* gene (data not shown).

PCR markers designed for other genes, RG556 linked to *xa-5* and RG136 linked to *xa-13* (Huang et al. 1997). Amplification of DNA genomic from 166 local accessions and 25 parents of hybrid rice with RG556 did not produce any polymorphism. The PCR products were therefore digested with *Dra*I endonuclease restriction enzyme (specific amplicon polymorphism was done by Huang et al. 1997) but were not successful in obtaining polymorphism (data not shown). The PCR marker linked to *xa-13* allowed efficient screening of 166 local accessions (fig. 1) and 25 parents of hybrid rice (fig. 2). The primers RG136 produced a resistant banding pattern with the size of 1500 bp (as IRBB13) and the other susceptible band 1000 bp in size (IR24). Five of 166 local accessions were detected carrying 1500 bp resistant band that assumed to contain *xa-13* gene (fig. 1). They are Nep Than (Acc. 8) in lane 3, Bong Sen (Acc. 95) in lane 9, Chet Cut (Acc. 88) in lane 11, Nep Mo (Acc. 189) in lane 15, and Nang Tra (Acc 199) in lane 19. Similarly, 3 of 25 parent lines of hybrid rice were selected because their banding pattern was the same as

that of IRBB13. Parent lines Son Thanh restorer in lane 11, IR66897B (maintainer) in lane 16, and IR75601B (maintainer) in lane 23 were assumed to contain *xa-13* gene (fig. 2).

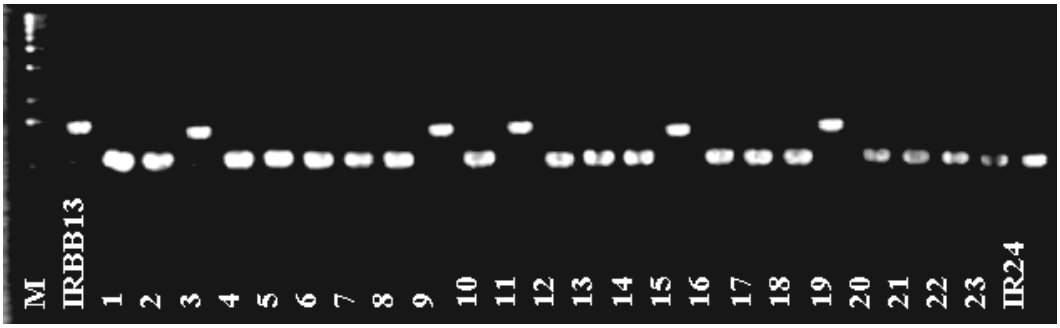

Fig. 1. PCR products of local rices amplified with primers derived from RG136. Lane M: the molecular weight marker. Lane 3: Nep than, lane 9: Bong sen, lane 11: Chet cut, lane 15: Nep mo, lane 19: Nang tra.

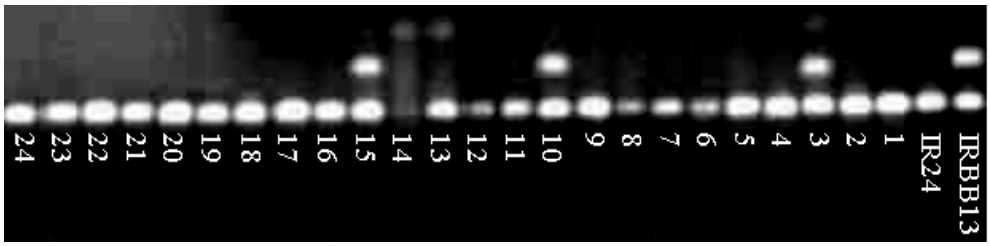

Fig. 2. PCR products of hybrid rices amplified with primers derived from RG136. Lane 3: IR75601B, lane 10: IR66897B, lane 15: Son thanh (R).

Reaction of local rices and parent lines of hybrid rice to bacterial blight

In the experiments screening for BB resistance, we always conducted including standard varieties. The reaction of check varieties was consistent with their spectrum and level of resistance to BB pathogen (table 1). Each of them carrying their own genes showed resistance reaction to races of *Xoo* that were resistant spectrum of these genes. The susceptible cultivar IR24 was not resistant to any races of *Xoo* (table 1). In the same condition and methods to screen 166 local accessions and 25 parent lines of hybrid rice in compare with standard varieties, their reaction were greatly various depending on groups. In general, local rices presented reaction ranged from highly susceptible to resistance to each of *Xoo* races. In 166 local accessions, just only 2 accessions (Lun Vang La Nho and Soi Da) were resistant to all 9 races, 3 accessions (Nang Ret, Tau Nguyen and Nang Huong resistant to 8 races, and 17 accessions resistant to at least 7 races of *Xoo* (table 2). The others were moderately susceptible with shorter lesion length than the susceptible check (data not shown). Five accessions assumed to contain *xa-13* gene were resistant to races No.4 and No.6 as the standard variety IRBB13 was. Screening for BB pathogen of 25 parent lines of hybrid rice showed their resistant spectrum to 10 *Xoo* races were various. Just only one maintainer line (Nhi32B) was resistant to all 10 races. A sterile line (Pea A1) and a restorer line (Son Thanh) were resistant to 9 races, 2 maintainer (IR75601B, and IR68885B) and a sterile line (IR78595A) were resistant to 8 races, 2 other sterile lines (BoA, IR73328A) and a restorer (Buc Khoi 838 R) were resistant to 7 races of *Xoo* (table 3). Three parent lines (Son Thanh R, IR66897B, and IR75601B) carrying *xa-13* gene were resistant to the races No.4 and No.6 as IRBB13 was.

Table 1. Resistant reaction of standard varieties to 10 *Xoo* races

No	Designation	Gene	*Xoo* races									
			1	2	3	4	5	6	7	8	9	10
1	IRBB4	*Xa-4*	R	S	S	R	R	S	R	R	S	R
2	IRBB5	*xa-5*	R	R	R	S	R	S	R	R	R	R
3	IRBB7	*xa-7*	R	R	R	S	R	S	R	R	S	S
4	IRBB10	*Xa-10*	S	R	S	S	R	S	R	S	S	S
5	IRBB13	*xa-13*	S	S	S	R	R	R	S	R	S	S
6	IRBB14	*xa-14*	S	S	S	S	R	S	S	R	S	S
7	IRBB21	*Xa-21*	R	R	R	R	R	R	R	R	S	S
8	IR24		S	S	S	S	S	S	S	S	S	S
9	Nang Som	*xa-13*	R	R	S	R	S	R	R	R	R	S

Note: R: resistant; S: susceptible

Table 2: Reaction of local rice accessions to 9 races of *Xoo*

No.	Acc.	Varieties	Gene	Reaction to 9 *Xoo* races									R/9
				1	2	3	4	6	7	8	9	10	
41	8	Nep than	*xa-13*	R	S	S	R	R	R	R	R	R	7
193	22	Nep tuong		R	S	R	R	R	R	R	R	S	7
199	28	Lun thong		R	R	S	R	R	R	R	R	S	7
201	29	Mot bui lun		R	R	S	R	S	R	R	R	R	7
128	30	Nep đau kim		R	R	S	R	R	R	R	R	S	7
129	32	Nep đo		R	R	S	R	R	R	R	R	S	7
72	55	Troi cho		S	R	R	R	R	S	R	R	R	7
78	61	Than nong mua		R	R	R	R	S	R	R	R	S	7
99	82	Lun vang la nho		R	R	R	R	R	R	R	R	R	9
105	88	Chet cut	*xa-13*	S	S	S	R	R	S	R	S	R	4
130	93	Soi bum bia		R	R	S	R	R	S	R	R	R	7
108	95	Bong sen	*xa-13*	S	S	S	R	R	S	S	S	S	2
164	165	Soi đa		R	R	R	R	R	R	R	R	R	9
167	172	Nang ret		R	R	R	R	R	R	R	R	S	8
174	180	Tau nguyen		R	R	R	R	R	R	R	R	S	8
177	184	Trang tep		S	R	R	R	R	R	R	R	S	7
179	187	Lem bui		R	R	R	R	R	R	R	S	S	7
181	189	Nep mo	*xa-13*	R	R	S	R	R	R	R	S	S	6
183	191	Nang huong		S	R	R	R	R	R	R	R	R	8
191	199	Nang tra	*xa-13*	R	R	S	R	R	R	R	R	S	7

Note: R: resistant; S: susceptible
R/9: number of 9 races that varieties resisted

Table 3. Reaction of hybrid rice lines to 10 races of *Xoo*

No.	Designation	Gene	Reaction to 10 *Xoo* races										R/10
			1	2	3	4	5	6	7	8	9	10	
1	Pea A1		R	R	R	R	R	R	S	R	R	R	9
2	IR68885B		R	R	R	S	R	R	S	R	R	R	8
3	Nhị 32B		R	R	R	R	R	R	R	R	R	R	10
4	Bo A		S	R	S	R	S	R	R	R	R	R	7
5	IR75601A		S	S	R	S	S	S	R	R	R	R	5
6	Nhị 32A		S	S	S	R	R	R	R	S	S	S	4
7	Bo B		R	R	R	R	R	S	S	R	S	S	6
8	IR78595A		S	R	R	R	R	R	S	R	R	R	8
9	IR68885B		S	R	S	R	S	R	S	S	R	S	4
10	IR73328B		S	S	S	S	R	R	R	S	S	S	3
11	Sơn Thanh (R)	*xa-13*	R	S	R	R	R	R	R	R	R	R	9
12	Minhhui 63 (R)		R	R	S	S	S	S	R	R	R	R	6
13	IR78595B		R	S	S	R	R	R	S	R	S	R	6
14	IR62829A		R	S	S	R	R	R	S	S	R	S	5
15	IR73328A		S	R	S	R	R	R	R	R	R	S	7
16	IR66897B	*xa-13*	R	S	R	S	R	R	R	S	R	S	6
17	RTQ5 (R)		S	R	S	R	S	R	S	S	R	S	4
18	559-14 (R)		R	S	S	S	R	R	R	S	S	S	4
19	968-1 (R)		S	S	S	S	S	S	S	S	R	R	2
20	Đac thanh (R)		R	S	S	S	S	R	R	R	R	R	6
21	R.23 (R)		R	R	S	S	S	S	S	R	R	S	4
22	IR75601B	*xa-13*	R	S	R	S	R	R	R	R	R	R	8
23	R242 (R)		R	S	R	R	S	S	S	R	R	S	5
24	Buc khoi 838 (R)		R	S	R	R	R	S	R	R	R	S	7
25	968-2 (R)		R	R	S	R	S	R	R	S	R	S	6

Note: R: resistant; S: susceptible;
A: cytoplasmic male sterile; B :maintainer ; (R): restorer
R/10: number of 10 races that varieties resisted

Marker-Assisted Selection for bacterial blight resistance rice

The *xa-13* gene among individuals of BC_1 population could not be detected. Therefore, BC_1F_1 plants were selfed to produce BC_1F_2 progeny to screen for phenotypic resistance and genotypic survey using 5 microsatellite markers. One hundred and thirty BC_1F_2 plants showing modern rice of IR24 phenotype were used to investigate their resistance to 10 *Xoo* races. Of these, 20 plants resistance to at least 6 *Xoo* races including race 4 and race 6 (typical resistance phenotype of *xa-13* gene) were genotyped for 5 microsatellite markers (RM21, RM114, RM122, RM164, and RM190). Polymerase chain reaction products of two markers RM21 and RM190 (of five markers) showed polymorphism. Amplification of DNA from these 20 plants with RM21 primers indicated that 11 plants showed resistant banding pattern as the donor (Nang Som) did (fig. 3A). Similarly, the amplification of DNA from the same 20 plants with RM190 primers detected 10 plants carrying resistant band of Nang Som (fig. 3B). Among plants carrying resistant banding pattern, 7 plants were found to contain both 2 markers (plants No. 5, 9, 11, 26, 34, 50, and 53). The RM21 marker was reported to locate in chromosome 11 (Panaud et al. 1996), while RM190 located in chromosome 6 (Temnykh et al. 2000). Hence, BC_1F_2 plants resistant to BB pathogen might carry different genes at various loci but not conferring to *xa-13* gene. The *xa-13* gene was not transferred from Nang Som variety to BC generations that carrying the modern rice of IR24 phenotype.

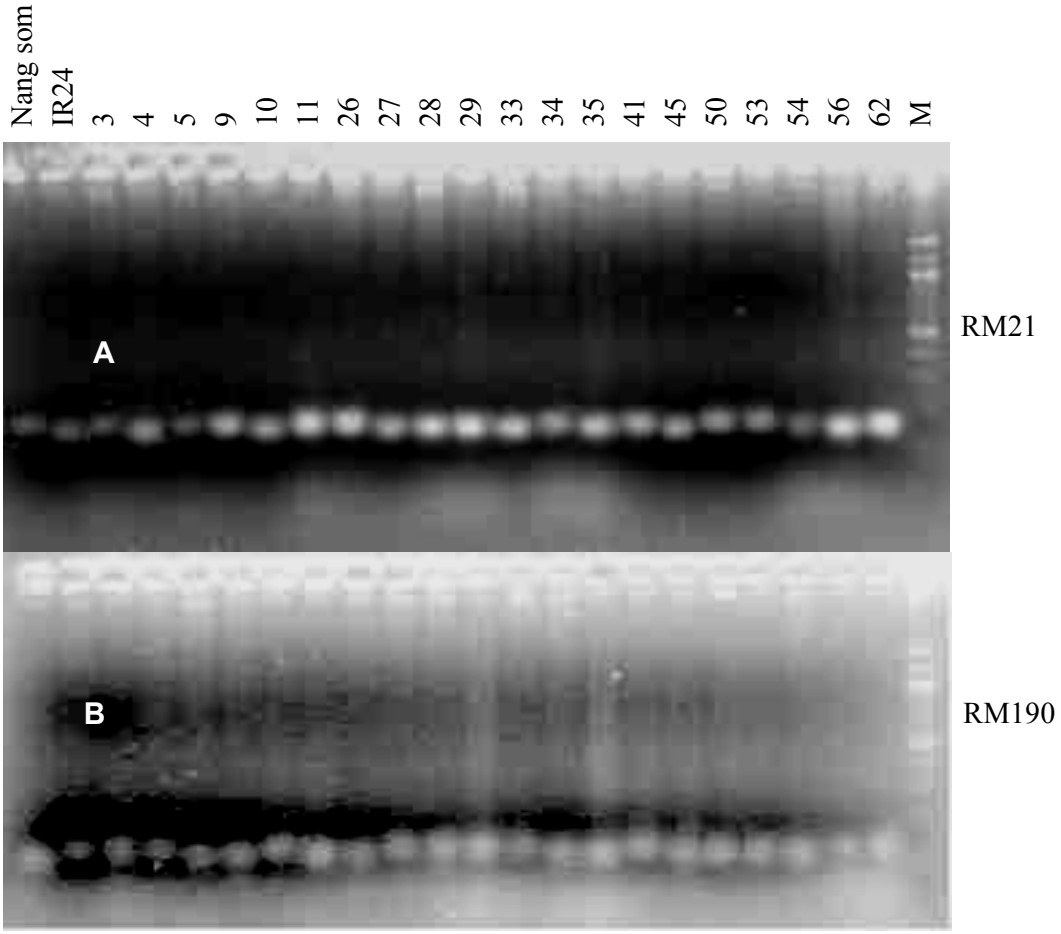

Fig. 3: PCR analysis of two markers loci with DNA from BC_1F_2 on agarose gel 3%. **A:** DNA amplified with RM21 primers. **B**: DNA amplified with RM190 primers. Lane M: the molecular weight marker.

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SUMMARY IN VIETNAMESE

Ứng dụng marker phân tử trong chọn giống lúa kháng bạc lá

Thí nghiệm khảo sát 166 giống lúa mùa, 25 dòng cha mẹ của lúa ưu thế lai sử dụng các cặp primers thiết kế từ RG556, RG136 và pTA248 để tìm các gen kháng *xa-5, xa-13* và *Xa-21*. Chúng tôi xác định được 5 giống lúa mùa và 3 dòng cha mẹ của lúa ưu thế lai có gen kháng *xa-13*. So sánh với thí nghiệm kiểu hình thanh lọc bệnh bạc lá cho thấy các giống có gen kháng *xa-13* đều cho phản ứng kháng với dòng vi khuẩn số 6 và số 4 như đối chứng IRBB13. Từ kết quả đa hình tìm gen kháng *xa-13* giữa giống lúa địa phương Nàng Sớm và giống lúa chuẩn nhiễm IR24, ta có thể phát triển các quần thể lai ngược IR24/Nàng Sớm để chuyển gen kháng *xa-13* và chọn lọc các cá thể con lai bằng marker thiết kế từ RG136.