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Molecular genetic analysis and marker assisted selection of bacterial blight resistance in hybrid rice

Nguyen Thi Lang and Bui Chi Buu

ABSTRACT

The objective of this study was to investigate the possibility of generating a polymerase chain reaction (PCR) – based polymorphic marker that can distinguish sterility and fertility. Based on the sequence data (STS) of RG140, pairwise primers were designed to amplify genomic DNA from IR62829A (CMS line) and IR46 (a restorer line) to identify polymorphic (*with* Eco RI and *Pvu* II *enzyme digestion*) *amplified products between the two rice lines. Two polymorphic markers, amplified with RG140FL/RG140RL, RG140FL/RB and RG532 FL/RL were identified. To examine the power of the identified specific amplicon polymorphic marker in predicting the genotypes of the F_2 individuals. These results demonstrate the utility of STS markers for use in marker-assisted selection and breeding within cultivated rice. Evaluate the reaction of two hybrid rice lines to 10 races of the BB pathogen. Bacterial blight (BB) caused by Xanthomonas oryzae pv. oryzae is one the most serious diseases of rice. Based on the sequence data (STS) of PB7, PB8, RG136 and RG566 pair wise primers which were is tightly linked to the bacterial leaf blight resistance genes Xa-21, xa-13 and xa-5 respectively, provides mean to perform marker-aided selection in a hybrid rice program in rice.*

Key words

Marker-aided selection, Molecular, RAPD, PCR, DNA sequencing, STS , CMS, gene cloning, *Xa-21, xa-5, xa-13*

INTRODUCTION

Full yield potential of hybrid rice is not realized because of the toll taken by the attack of diseases and insects. It is estimated that yield loss due to diseases and insects is up to 20-25% annually in Vietnam. The varietal resistance breeding is considered in an effective approach to eliminate the use of pesticides and minimize crop losses due to this disease. Recent applications of molecular marker are beginning to unlock the opportunities to exploit genetic information and maintaining more durable resistance genotypes in hybrid rice To facilitate the marker aided selection (MAS) technology to hybrid rice breeding programs in Vietnam and other Asian countries, PCR-based DNA markers have been developed for precise and efficient transfer of the CMS restoring gene into new elite restorer lines. This was done with an informative RFLP marker RG140, and RG532 which showed the closest linkage to restoring *Rf-3* in rice to provide means to perform marker-aided selection in rice program. The disease cause yield losses through reduced reduced tillering, unfilled panicles. In Vietnam , bacterial blight has been the most rice disease.

Recently, the concept of marker-assisted selection (MAS) have provided excellent discussion on the theory and advantages of using molecular marker-based selection for crop improvement over selection based solely on the phenotype (Tanksley et al. 1989, Paterson et al. 1991). It may provide new solution for selecting and maintaining more durable important genes in rice. Marker-aided selection is also helpful in attempts to transfer genes from exotic germplasm into cultivated line. Especially, the blast resistance gene mapped in rice *Pi-2(t)* in chromosome 6 is located 2.8cM distant from the RFLP marker RG64 (Yu et al 1991), providing an opportunity to initial identify rice blast resistance gene *Pi-2(t)* (Hittalmani et al. 1995). The identification of the resistance

gene *Xa-21* in F_2 population was based on the closely linked marker pTA248 (Ronald and Tanksley 1991), providing development PCR based for MAS (Chunwongse et al. 1993). Rapid progress has been made in gene mapping, development of molecular markers and genome comparisons. However, reports on actual use of marker-aided gene transfer are very scant. One such work is the molecular mapping and marker-assisted selection for the semi-dwarf gene, *sd-1* (Cho et al., 1994)

In the hybrid rice, several genes for male sterility and fertility restoration have been linked with DNA markers. One TGMS gene (*tms-1*) of the line 5460S from the China is located on chromosome 8 (Wang et al 1995). TGMS gene *tms-2* of Norin PL12 from Japan. One of the TGMS gene *tms-3(t)* have been mapped with random amplified polymorphic DNA(RAPD) on chromosome 6 (Shubudhi et al. 1996; Lang et al. 1997). The identificantion of *Rf-3* nuclear ferlity-restoring gene for WA-CMS is located on chromosome 1 (Zhang et al. 1996), providing development PCR based DNA markers for the CMS fertility restoring gene *Rf-3* in rice (Lang et al. 1998).This is providing an opportunity to be very useful for efficient marker assisted selection in rice breeding.

MATERIALS & METHODS

Plant material

A molecular genetic survey was conducted to find PCR-based markers that show polymorphic detection between DNAs from CMS line IR62829A, and a donor restorer line IR46. Another population BC_1F_1 from IR 66897A / IR66897B was also considered. IR62829A, and IR66897B were developed in IRRI and applied in Cuulong Delta Rice Research Institute to produce F_1 seeds with appropriate restorers.

Rapid isolation of rice DNA

Isolate DNA for PCR analysis which does not require liquid nitrogen, needs only small amount of tissue sample. Protocol was done according to the method suggested by Zheng et al. (1995). DNAs suitable for PCR analysis were prepared using a miniscale procedure in a labeled 1.5 ml centrifuge tuble in ice. The young leaf was ground using a polished glass rod in a well of a Spot Test plate (Thomas Scientific) after adding 400 μl of extraction buffer (50 mM tris-HCl pH 8.0, 25 mM EDTA, 300mM NaCl and 1% SDS). Ginding was done until the buffer turned green which is an indication of cell breakage and release of chloroplasts and cell contents. Another 400μl of the extraction buffer was added and mixed into the well by pipetting. 400μl of the lysate was transferred to the original tube of the leaf sample. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA precipitated using absolute ethanol. DNA was air-dried and resuspended in 50μl of TE buffer (10mM Tris- HCl pH 8.0, 1mM EDTA pH 8.0).An aliquot of 1μl is sufficient for PCR analysis. DNA quality and quantity were spectrophotometrically determined.

PCR amplification

The genomic DNA from both of F_2 plants and parents were subjected to PCR amplification using the synthesized primers. The PCR buffer consisted of Tris pH 8.4[10mM], KCl [50mM], $MgCl_2$[1.8mM] 0.01mg/ml gelatin. *Taq* [5 unit of *Taq* polymerase in a volume of 25μl]. Template DNA were initatially denatured at 94°C for 5 minutes, followed by 30 cycles of PCR amplification under the following parameters, 1 min. for denaturation at 94°C, 1 min. for primer annealing at 55°C and 2 min. for primer extension at 72°C. Final 5 min. for incubation at 72°C was allowed for completion of primer extension on a 480-thermal cycler. The amplified products were electrophoretically resolved on 1.2 % agarose and using 1xTAE buffer.

Restriction digestion of PCR products

When combinations of primer RG 140FL/RL and RG140FL/RB were used to amplify the DNA of the parents IR62829A and IR46, the PCR products were monomorphic. Therefore, restriction enzymes were used to generate SAP fragments (as described by Williames et al. 1991). The amount and quality of PCR amplification was first monitored by running 10μl aliquots of the reaction mixture on an agarose gel. The

enzyme mix 3.2 μl sterile distilled water, 1.5 μl restriction buffer (10X), 0.3 μl restriction enzyme (10U/μl). After a brief spin in a microfuge and overnight or 4 hours of incubation at 37°C, the digested products were run on 1.5% a agarose to resolve digested PCR fragments.

Reaction to bacterial blight

Parental lines were inoculated with the 10 Philippine races of *Xanthomonas oryzae* (*Xoo*) to determine the reaction of hybrid rice and to confirm the reaction of donor lines.

RESULTS

Sequencing RG140 and designing PCR primer .

The nucleotide sequence of RG140 was determined on the basis of the orientation to ensure accuracy. The present G+C content of the clone was determined to be 50-60% (fig.1)

Figure 1: DNA sequence of RG140

F

1	GAGCTCGCCC	GGGGATCCTC	TAGAGTCGAC	CTGCAGGTAG	CTGGTCAAAT
51	CCTCAGGTGA	TTTTGTTAGC	TCATATTTTG	GTAAATAATT	**CCATATGTAC**
101	**ATAGTAGCAC**	**CTGCTCCACC**	ATTTGAGTGG	AGTGTCATAT	ATAGGTGAAG
151	TACTTGAATT	ACTCACTTCG	TCCCAAAATA	AGTGCAGCTG	TGTAGTTCAC
201	CGGTTGCACT	TATTTTAGGG	TGGAAGGAGT	ATATATTAAG	CATTATTGGG
251	AAGATGATTT	CCTCCTAATC	CACAAGTTTT	TTTTCACAAG	TTTATATGTT
301	ATACC				

R

1	GCCAAGCTTG	GGCTGCAGGG	CCACACCCAC	ATATGCCCAT	GCACATCCAT
51	CCATCGTCCG	GCTTCACCTA	TGGTTTTTCA	GATTCACTTC	ACACA**TCCCT**
101	**AGTTTGTGCT**	**ACTCCTAGCT**	ACAAATTGAA	ATTCCATATT	GGCATCTCAT
151	TGTTCATTTC	AGGCCTCAAT	ATTGGGCCCT	CCAGCCAGCC	AGTACGTATG
201	TCCTAGTGTC	TCATGCTAFT	CAACAATGAC	AGGACATTGG	TAAGTTACCC
251	AGTCTGTCTG	TTCTGAAGAC	ACAGGAATTT	TACAGCATTT	TTACACACAA

Digestion of PCR products with restriction endonuclease has been found to increase the level of polymorphism as well as the number of alleles in rice (Willians et al. 1991). We are performing STS analysis in a cross IR628295A / IR46R. It is clear from fig. 2 that PCR can detect size for polymorphism at certain loci. For example, amplification of DNA from IR628295A and IR46R with the primer RG140FL / RL gave rise to 1400bp products. *Eco*RI digestion revealed a polymorphism between IR628295A and IR46R. When combination pair of the primer RG140FL / RL, two restriction sites for *Eco*RI are present in PCR product of IR46 with size 900bp and 500bp and only one band for IR628295A. This polymorphism can be used as marker to distinguish between *Rf3* and *CMS*, because all loci were scored as codominant.

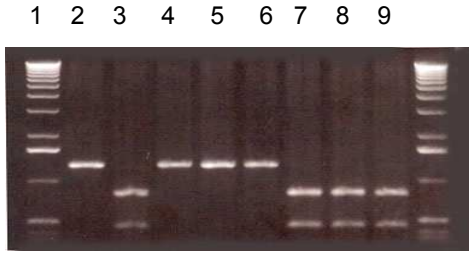

Figure 2: PCR product amplification after digestion by enzyme *Eco*RI

Lane 1: M , 2: IR628295A 3: IR46R, 4: ZSA, 5:IR58025A 6: IR68897A, 7: IR24R, 8: IR40750-82-2-2-3R, 9: IR 36R

In case of RG532L-L, combination of primer RG532FL/RL showed no polymorphism between IR46 and IR628295A. Restriction enzymes digested PCR products were used as *Alu*I *and Rsa*I. *Rsa*I fragments are identifical between IR628295A and IR46R, they have to be scored as dominant or recessive due to absence of a band in one of the parents. For example, three restriction sites for *Rsa*I are present in PCR product of IR46R (350 and 250bp), and IR628295A (800, 350 and 250bp) (Figure 3).

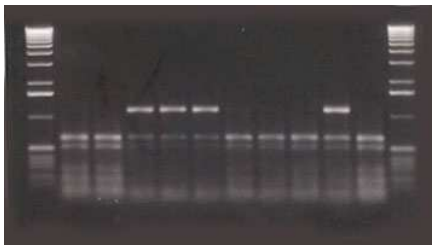

Figure 3: PCR amplification of the RG532 FL/RL after digestion by *Alu*I

Lane 1: M, 2: IR 24R 3: IR46R, 4: IR628295A, 5:IR58025A 6: IR68897A, 7: IR40750-82-2-2-3R, 8: IR 36R,IR 68897 A, 9:IR IR66897A and 10: IR IR66897B

Germplasm survey of polymorphism

Germplasm classification as a potential of using a PCR-based approach to detect DNA polymorphism for rice, was implemented. When genomic DNA of the various genotypes IR24R, IR40750-82-2-2-3R, ZSA, IR58025A and IR68897A were PCR amplified using the above primers combinations, they showed no polymorphism. However, when PCR products were digested by *Eco*R1 and *Pvu*II enzymes with primers RG140FL/RL, the results distinguished polymorphic bands from IR24R, IR40750-82-2-2-3R. They have the same band with IR46R. Otherwise, IR58025A, IR68897A, ZSA have the same band with IR628295A. Therefore, a PCR-based RFLP linked to the *Rf-3* is generated between restorer and sterile parents.

In case of RG532, the genomic DNA of genotypes such as IR46R, IR40750-82-2-2-3R, IR58025A, ZSA and IR68897A were used as templates. There were polymorphic bands among varieties in order to compare the

segregation pattern of the SAP marker with that of RFLP.

Analysis of F_2 population

DNAs from the F_2 population, one of the cross between IR628295A x IR46, were assessed through PCR amplification using primer RG140 FL/RL. The resultant PCR products were spliced out by enzyme digestion *Eco*RI. Fragments were resolved on agarose gels and the banding patterns were scored with reference to those of the parents. The banding pattern of the F_2' s individuals could be classified into homozygote for the IR46R type marker size 900bp and 500bp fragment, homozygote for IR628295A type marker size 1400 bp fragment, and heterozygotes [displaying both fragments IR46R and IR628295A) (fig4). Similarly, when combinations of primer pair RG140L-B, fragments were resolved on agarose gels and the banding patterns were scored with reference to those of the parents. The banding pattern of the F_2' s individuals could be classified into homozygote for the IR46R type marker 800bp and 400bp fragments, homozygote for IR628295A, type marker 1200 bp fragment, and heterozygotes [displaying both fragments IR46R and IR628295A). Southern blots of F_2 population were probed with radiolabeled RG140. The segregation pattern of the RFLP marker was the same as that of the SAP marker, indicating that the same locus was detected by both procedures (Figure 5)

Chi-square test of goodness-of-fit suggested close agreement of the SAP and RFLP markers with segregation of the expected Mendelian 1:2:1 ratio

Selection based on a single marker

Closely linked DNA markers have been identified for *Rf-3.* A single marker to identify *Rf-3* in a segregating population in 120 F_2 population was based on a closely linked marker RG140FL/RL from IR628295A/IR46R. The results of two approaches are compared in table 1. Of the 20 individual plants have been scored as homozygous *rf3 rf3* based on the SAP and RFLP marker, 20 were found to be homozygous upon progeny testing. This give an accuracy of 100%. Of 79 plants that were scored as heterozygous *Rf3 rf3* based on progeny testing, 74 plants were found to be heterozygous the SAP and RFLP markers. Three plants were found to be homozygous *rf3 rf3* and two plants were found to be homozygous *Rf3 Rf3*. This gives an accuracy of 93.6%, 21 plants, were found to be homozygous *Rf3*, when identifyting homozygous restore genotypes. 100% was obtained, this gives an accuracy of 90,4%.

Table 1 *Rf-3* analysis of F_2 through progeny test and PCR from IR2829A/IR46

Progeny test		PCR analysis			Accuracy (%)
Genotype	Number of plants	*rf3*	*Rf3rf3*	*Rf3*	
rf3 rf3	20	20	0	0	100.0
Rf3rf3	79	3	74	2	93.6
Rf3Rf3	21	0	2	19	90.4

Selection based on flanking markers

Single marker based selection can be corrected if flanking markers are used for marker-aided selection. We used both the flanking markers, RG 140FL-RL and RG532 FL-RL. Selection accuracy was 100% in identifying with homozygous sterility plant from a segregation of F_2 population from the cross of IR628295A /IR46R (table 2).

Table 2: Prediction of genotypes of F_2 plants based on the flanking RFLP marker (RG140FL/RL and RG532 FL/RL) for the *Rf-3* locus

Prediction		Progeny testing		Accuracy (%)
RG532	RG140	*rf3*	*Rf3*	
rf3(20)	*rf3*(20)	20	0	100
Rf3(21)	*Rf3*(21)	0	19	100

Reaction of hybrid rice lines to bacterial blight

The reaction to 10 races of *Xoo* of the 24 breeding materials of hybrid rice is presented in table 3. Pea A showed its resistance to 9 races except race 7. IR 75601B and IR 6697B showed their resistance to 7 races except race 1, race 2, and race 3. Another breeding materials were some resistant and some susceptible.

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

Designation	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6	Race 7	Race 8	Race 9	Race 10
Pea A	R	R	R	R	R	R	S	R	R	R
Nhi 32	R	R	S	S	R	R	R	S	S	S
Nhi 32A	S	R	S	R	R	R	R	S	S	S
Bo A	S	R	S	R	S	R	R	R	S	R
IR 75595 A	S	S	R	S	R	R	S	R	R	S
IR75601A	S	S	R	R	S	S	R	R	R	R
IR 73328A	S	R	S	S	R	R	R	R	R	S
IR 62829A	R	S	S	R	R	R	S	S	R	S
Son Thanh	R	S	R	R	R	R	R	R	R	R
Nhi 32B	R	S	R	R	R	R	R	R	R	S
BoB	R	R	R	S	R	S	S	R	S	S
IR78595B	R	S	S	R	S	R	S	R	S	R
IR75601B	S	R	S	R	R	S	R	R	R	R
IR68885B	S	R	R	S	R	R	S	R	R	S
IR73328B	S	S	S	S	R	R	R	S	S	S
IR66897B	S	S	S	R	R	R	R	R	R	R
IR68885B	S	R	S	R	S	R	S	S	R	S
IR73328B	S	S	S	S	R	R	R	S	S	S
IR66897B	R	S	R	S	S	R	S	S	R	S
IR68885B	S	R	S	R	S	R	S	S	R	S
IR62829A	S	R	S	R	S	R	S	S	S	S
IR46 R	S	S	S	R	S	S	S	S	R	S
IRBB21	R	R	R	R	R	R	R	R	R	S
IR24	S	S	S	S	S	S	S	S	S	S
IRBB5	R	R	R	S	R	S	R	R	R	R
IRBB13	S	S	S	R	R	R	R	R	R	R

R: resistant

S: susceptible

R and S reactions are expressed in lesion length as R < 5cm, Intermediate = 5-10cm, Susceptible >10cm

Similarly, one population including 59 BC_1F_1 lines from IR668697A / IR66897B indicated resistant and susceptible scores to these races. However, in case of IR628295A/IR 46R, no plant was recorded all

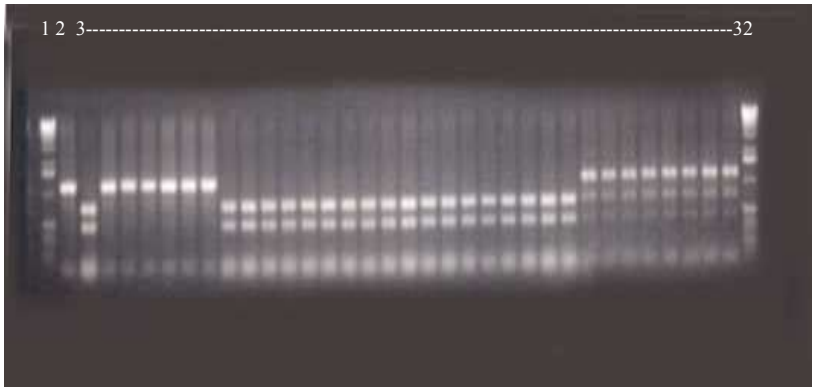

Figure 4: PCR products in segregating population were generated by DNAs with primer RG140FB/RL. Lane 1: IR628295A, lane 2: R 46R, lanes 3-32 : F_2's individuals indicating the three resistances gene was obtained. The disease reaction of the 59 plants were verified by inoculation with 10 races of the bacterial blight pathogen.

PCR primers for *xa-5* were designed based on RG556 sequence tag site, the 22 breeding materials were susceptible all for both *xa-5* and *Xa-21.*

PCR primers for *xa-13* were designed based on RG136 sequence tag site, to amplify DNA from IR66897B and IR75601B. The primer pairs RG136 produced a 1500bp resistant DNA band for IR66897B and 1000bp for six supceptible cutters (Figure 5).

Hybrid rice lines resistant to bacterial blight

Plants containing *xa-13* resistance gene (identified through molecular markers) in various combinations were selected in the BC_1F_1 population. Using a selective genotyping strategy combined with parental survey, the DNAs closely linked markers on chromosome 8, RG136FR, were found related to bacterial leaf blight resistance (*xa-13*). Fifty nine plants in BC_1F_1 showing the CMS phenotype and possessing one resistance gene (*xa-13)* were obtained. Table 4 shows the marker genotypes and the disease reaction of the 20 BC_1 F_1 plants containing resistance gene *xa-13.*

Table 4:The segregation of molecular marker (RG136F/R) in parents and 20 individuals of the cross IR66897A/ IR66897B.

Designation	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6	Race 7	Race 8	Race 9	Race 10	RG 136
IR66897B	S	S	S	R	R	R	R	R	R	R	2
IR66897A	R	S	S	S	S	R	S	S	R	S	1
1	S	S	S	R	R	R	I	I	S	S	1
2	R	S	R	R	R	R	R	R	R	S	1
3	R	R	R	S	R	S	S	R	S	S	1
4	S	S	S	R	R	R	R	R	R	R	2
5	S	S	S	R	R	R	R	R	R	R	2
6	S	S	S	R	R	R	R	R	R	R	2
7	S	S	S	S	R	R	R	S	S	S	1
8	S	S	S	R	R	R	R	R	R	R	2
9	S	S	S	R	R	R	R	R	R	R	2
10	S	S	S	R	R	R	R	R	R	R	2
11	R	S	S	S	S	I	S	S	I	S	1
12	R	S	S	S	S	R	S	S	I	S	1
13	R	S	S	S	S	R	S	S	R	S	1
14	R	S	S	R	I	I	S	S	R	S	1
15	R	S	R	R	R	R	R	R	R	R	1
16	S	S	S	R	R	R	R	R	R	R	2
17	R	S	S	S	S	R	S	S	R	S	1
18	S	S	S	R	R	R	R	R	R	R	2
19	R	R	S	R	R	S	R	R	R	R	1
20	S	S	S	R	R	R	R	R	R	S	2

1: band size 1.0 kb
2: band size 1.5 kb
R and S reactions are expressed in lesion length as R < 5cm, Intermediate = 5-10cm, Susceptible >10cm

DISCUSSION

Two RFLP markers RG532 and RG140, were mapped on chromosome 1 and found to be closely linked to *Rf-3* for fertility restorer in hybrid rice program. The RFLP insert clone into plasmid pUC8 and PGEM4Z vectors was sequenced. One RFLP marker RG136, was mapped on chromosome 8 and found to be closely linked to *xa-13* for bacterial leaf blight resistance. Double stranded DNAs were isolated and used as template for dideoxy chain termination conversion into STS will provide means to perform marker-assisted selection. The capability of PCR to amplify specific nucleotide sequences as million times to facilitate the use of technologies in terms of of DNA analysis that would be difficult with conventional molecular biology techniques. The amplified fragments can be visualized on ethidium biomide stained agarose gels, hence genomic sequences can be rapidly detected without hybridization analysis. It is clear from fig. 4 that PCR-based can detect polymorphism size at certain loci especially when DNA from distantly related plants are amplified. In case of genotypes ZSA, IR24, IR46..., none of the amplified loci directly show polymorphism size. We have consequently digested the amplification products with several four nucleotides recognizing restriction endonuclease

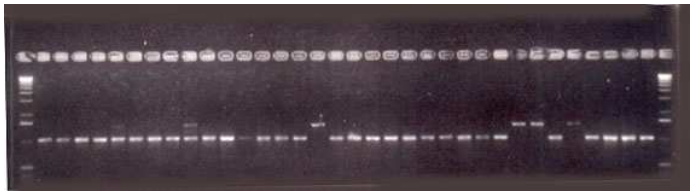

Figure 5: PCR products in BC_1F_1 from IR66897A/ IR66897B were generated by DNA with primer RG 136F/R. The primer pairs RG136 produced a 1500bp resistant DNA band for IR66897B and the size 1000bp for 6 supceptible cutters to detect base. It is show that PCR products were then digested with 6 base reconizing restriction endonucleases to detect DNA variation not detectable as ALP

In theory, this minor inaccuracy in single marker-based selection can be corrected if flanking markers are used for marker-aided selection. We tested this with the restorer gene *Rf-3,* which has been previously mapped with RFLP marker RG532 (Zhang et al 1996) . We found a flanking marker, RG140, and used both the flanking markers, RG140 and RG532, to perform marker-aided selection. Selection accuracy was 100% in identifying homozygous sterility and fertility plants from a segregating F_2 population. Similarly, the closest STS marker was RG136 concerning *xa-13* gene from another BC_1 F_1 population from IR 66897A/ IR66897B. These results demonstrated the usefulness of marker-assisted selection to precisely identify the genotype of a linked target gene in a segregating population. Especially, when the selected plants are to be used for futher crosses. PCR methodology provides a more effective means of performing marker-aided selection than screening by RFLP and PCR analysis is less expensive than RFLP analysis because of the lower cost of reagents and the reduction of time and labor required (Penner et al 1995). PCR-based markers have opened up a new avenue to map genes of economic importance. The mapping of gene *Rf-3* provided the oppoturnity for marker-assisted selection in hybrid rice.

The successful transfer of *xa-13* to the hybrid rice by using MAS demonstrate the effectivess of MAS for bacterial blight resistance breeding. The accuracy of marker-aided selection for *xa-13* was verified through BC_1F_1. The accuracy of predicting homozygous resistant genotypes based on marker data was 98% for *xa-13*.

The tools of molecular biology now provide the opportunity to develop large numbers of phenotypically neutral genetic markers in any organism from which DNA can be extracted. The significant progress in the introgression of exotic germplasm into well – adapted cultivars is also anticipated. The use of PCR technology to rapidly amplify genomic DNA fragment will be a significant approach.

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SUMMARY IN VIETNAMESE

Phân tích di truyền tính kháng bệnh bạc lá của lúa ưu thế lai

Thí nghiệm nhằm mục đích tìm gen kháng bệnh bạc lá với các nòi có độc tính cao ở Đông nam Á trong các vật liệu được sử dụng để sản xuất lúa lai hệ thống 3 dòng. Trên cơ sở dữ liệu chuỗi ký tự của RG140, chúng tôi thiết kế cặp mồi để tìm kiếm gen bất dục đực tế bào chất của IR62829A và dòng phục hồi phấn hoa IR46, với sự phân cắt của enzyme *EcoRI* và *PvuII*. Hai marker có tính đa hình RG140FL/RG140RL và RG532 FL/RL đã được phân lập. Trên cơ sở dữ liệu chuỗi ký tự của PB7, PB8, RG136 và RG566, các cặp mồi tương ứng đã được thiết kế nhằm phát hiện gen kháng *Xa-21*, *xa-13* và *xa-5*.