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GENETICS AND BREEDING FOR BLAST AND BACTERIAL LEAF BLIGHT RESISTANCE OF RICE (*Oryza sativa*. L)

Nguyen thi Lang[1] Trinh thi Luy[1], Bui thi Duong Khuyeu[1] and Bui Chi Buu[2]

1: Cuu Long Delta Rice Research Institute, CanTho, Vietnam (CLRRI)
2: Institute of Agricultural Sciences for Southern Vietnam (IAS)

ABSTRACT

Genetics for bacterial pathogens are difficult to manage. Development of host plant resistance is the most effective means of disease management. As many as genes conferring resistance (bacterial blight) and some genes for resistance to blast have been identified to various races of the pathogen have been identified and utilized in rice breeding programs. To evaluate the genetic backgrounds and introgression of resistance genes from donor varieties, the graphical genotypes of the population were constructed using 3 simple sequence repeats RM44, RM111, RM 483 and φX174 – Hae III. However, large-scale and long-term cultivation of varieties carrying a single gene for resistance resulted in a significant shift in pathogen race frequency with consequent breakdown of resistance in these cultivars. To combat the problem of resistance breakdown, pyramiding of resistance genes into different cultivars is being carried out. Pyramiding of resistance genes is now possible with molecular markers that are developed for individual genes. This report the gene for biotic stress such as bacterial blight, blast resistance, brown plant hoper genes identified and their corresponding molecular markers developed for breeding durable resistance into modern rice.

Keywords: Bacterial blight, blast, *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

Mekong Delta is the biggest granary of Vietnam. It produces more than 50% of rice in the country. Intensive cultivation of rice in the delta has led to increased threat due to continuous changing disease races and insect biotypes. Moreover, a large part of the delta is severely affected by the acid sulfate soil conditions. Thus, pests and disease stresses lower rice productivity in Mekong Delta. Breeding disease resistant genotypes using marker assisted selection in rice have reported (Lang et al. 2004).One of the main objectives of plant breeders is to improve existing cultivars, which are deficient in one or more traits by crossing such cultivars with lines, which possess the desired trait. A conventional breeding program thus involves crossing whole genomes followed by selection of the superior recombinants from among the several segregation products. Such a procedure is laborious and time consuming, involving several crosses, several generations, and careful phenotypic selection. In addition, tight linkage of the desired loci with undesired loci may make it difficult to achieve the desired objective. Recombinant DNA methodology can help to overcome a few limitations, but genetic engineering approaches are also limited by the lack of sufficient number of cloned genes and the lack of availability of standardized transformation protocols in many crop species. Moreover, polygenic traits are difficult to manipulate by genetic engineering procedures. To overcome these constraints limiting rice production in Vietnam, There is urgent need to widen the gene pool of rice cultivars cultivated in Mekong Delta. Fortunately, wild species of rice are an important reservoir of useful genes to meet these challenges. This report elaborates on the applications of DNA marker technology in the genetic analysis and, the markers, which can be employed in MAS for breeding varieties and hybrids with durable bacterial blight resistance, blast resistance in rice.

The rice-rice blast interaction has long served as a model system to study plant-pathogen interaction

(Valent 1990). Two new resistance genes were reported *Pi-36* on chromosome 8 (Liu et al. 2007) and *Pi-37* on chromosome 1 (Lin et al. 2007). After that, a novel gene *Pi-40* linked to the DNA markers derived from the NBS-LRR motifs confers broad spectrum was reported in Korea (Jeung et al. 2007).

Currently, about 30 resistance genes or loci against to *Xoo* have been identified in cultivated and wild rice (Gu et al. 2008). So far, five dominant *R* genes, *Xa-21* (Song et al. 1995), *Xa-1* (Yoshimura et al. 1998), *Xa-26* (Sun et al. 2004), *Xa-27* (Gu et al. 2005) and *Xa-3* (Xiang et al. 2006) and two recessive *R* genes, *xa-5* (Iyer and McCouch 2004) and *xa-13* (Chu et al. 2006), have been isolated by map-based cloning. Long arm of chromosome 11 of rice is rich in *R* genes for disease resistance to bacterial blight: *Xa-10, Xa-3, Xa-4, Xa-21, Xa-22, Xa-23, Xa-26* (Gu et al. 2008). Among them, *Xa-21, Xa-26, Xa-3* have been isolated and all of them encode leucin-rich repeat (LRR) receptor kinase-like proteins (Song et al. 1995, Sun et al. 2004, Xiang et al. 2006). *Xa-3* is allelic to *Xa-26* and they share 92% sequence identity. *Xa-3/Xa-6, Xa-4* and *Xa-22*(t) were mapped to the distal end of chromosome 11L (Gu et al. 2008). *Xa-21* and *Xa-23* reside at the middle region of chromosome 11L. Both *Xa-21* and *Xa-23* conferred broad spectrum resistance to multiple *Xoo* strains, including six races of the Philippines strains (Khush et al. 1989, Zhang et al. 2001)

MATERIALS AND METHODS

Plant materials

Microsatellites RM21, RM162, RM483 and one STS marker RG64F-R were used to study rice blast

Near isogenic lines IRBB5, IRBB53, IRBB10, IRBB14, IRBB13, IRBB21 check and their recurrent parent IR24 were used in these studies. Twenty five lines were used for selection of BB resistance lines. Local and improved varieties developed from the selected, special compatible lines were used to analyze the presence of the number of alleles for the resistance genes *xa-5, xa-13* and *Xa-21*. Near isogenic lines, IRBB4 (*Xa-4*), IRBB5 (*xa-5*), IRBB13 (*xa-13*), and IRBB21 (*Xa-21*) carrying specific bacterial blight resistance genes and their recurrent parent, IR 24 were used for allele designation. More than 1800 accessions of traditional rice were collected and evaluated for rice breeding at the CLRRI's gene bank.

The plants were grown in the field. DNAs were extracted from rice leaves.

DNA isolation

A crude DNA preparation suitable for PCR analysis was prepared using a simplified miniscale procedure (Lang 2002). A single piece of healthy youngl leaf was harvested and placed in a labelled 1.5 ml centrifuge tube in ice. The leaf sample was macerated using thick glass rod after adding 400 μl of extraction buffer (50 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 300 mM NaCl and 1% SDS). The leaf was grounded until the buffer turned into green colour. After grinding, another 400 μl of extraction buffer was added and mixed by pipetting. The contents were centrifuged at 12,000 rpm in micro centrifuge for 10min. Nearly 400 μlof lysate was extracted with 400 μl of chloroform. The top aqueous supernatant was transferred to another 1.5ml tube and DNA was precipitated with absolute ethanol. The contents were centrifuged for 3 min at full speed and the supernatants were discarded. The pellet was washed with 70% ethanol. The DNA was air driedand re-suspended in 50 μl of TE buffer (10mMTris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). One ml of aliquot was used for PCR analysis and the remaining solution was stored at –20°C for any further use.

Polymerase chain reaction (PCR amplification)

A series of optimization experiments using parents and isogenic samples was carried out. In which concentrations of template DNA, primers, dNTPs and *Taq* polymerase were varied to determine given conditions. The PCR reaction mixture of 20 μl contained 25–50 ng template DNA, 50 ng of each primer obtained from CLRRI, 0.05 mM dNTPs, 1X PCR buffer (10 mMTris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2and 0.01% gelatin) and 1 unit of *Taq* polymerase obtained from Lang, Genome mapping Laboratory, CLRRI. The template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 1 min denaturation at 94°C, 1 min primer annealing at 55°C and 1.5–2.0 min primer extension at 72°C. A

final 7 min incubation at 72°C was allowed for completion of primer extension on thermalcycler. Following amplification, the samples were run on a 1% agarose in 1X TBE buffer stained with 0.1μg/ml of ethidium bromide and visualized under ultraviolet light. For PCR-RFLP, initially 5 μl of PCR product was used for gel electrophoresis to determine successful of amplification. Seven to eight μl of the PCR product was then used for restriction enzyme digestion if the amplification was successful. The total reaction volume was usually 20 μl. The reaction mixture consisted of 11.4 μl of double distilled water, 2μl of 10 X restriction buffer, 0.6 μl restriction enzyme (10 U/μl) and 6–8 μl of PCR products. The reaction mixture was incubated at 37°C for 6–8 h. The DNA fragments digested by restriction enzymes were separated on 1.5% agarose gel and visualized under UV light after staining with ethidium bromide.

Bacterial blight, blast screening

The rice plants were inoculated at maximum tillering stage with the IRRI strains of *Xanthomonas oryzae* pv. *oryzae* (Xoo) and one race 11 of CLRRI were used in inoculation . All plants were grown in the greenhouse and inoculated at 55 days after planting. The leaf blades were inoculated by scissor clipping at 5 cm below the leaf tips, the plants were scored as resistant or susceptible genotypes by measuring the length of lesion or by visual scoring.

Blast evaluation: The inoculum was prepared as described by Bonman and Mackill (1988)

Statistical analysis

Chi square tests were performed to examine the goodness of fit between expected Mendelian radio, SSR and phenotype data for analysis.

RESULTS AND DISCUSSION

BREEDING FOR BLAST RESISTANCE

Differential varieties for rice blast resistance were used**.** The resistance genes in CLRRI bred rice varieties were examined based on reaction patterns of isolates of blast *Pyricularia grisea* Sacc. from CLRRI. To confirm genes, genetic analyses were carried out using BC_1F_2 progenies derived from crosses of IRRI varieties with IR24 and IR36. This study demonstrated the utility of differential system in elucidating the genetic constitution for blast resistance of CLRRI and IRRI varieties (Table 1).

To evaluate the genetic backgrounds and introgression of resistance genes from donor varieties, the graphical genotypes of the population were constructed using 3 simple sequence repeats (SSR marker), RM44, RM111, RM 483 with marker φX174 – *Hae III* (fig 1). These materials will be used in breeding program.

Table 1. Reaction patterns of F_2 lines from IR24 / OM2514 and IR36 / OM2514 to blast isolates from CLRRI (POM -*1)* (scoring from 0 to 9)

Designation	0	1	3	5	7	9
IR24						30
OM2514		30				
F_1	50	32	27	44	27	75
BC_1F_2	32	45	22	44	27	75
IR36 / OM2514						
IR36						10
OM2514		30				
F_1	50					
BC_1F_2	12	40	34	27	50	25

A set of differential varieties is a useful tool to identify blast resistance genes in rice and to characterize new promising varieties. The reactions of 28 lines were confirmed to one blast isolates of CLRRI, with primer RM44, RM111, RM483 with check φX174 – *Hae* III.

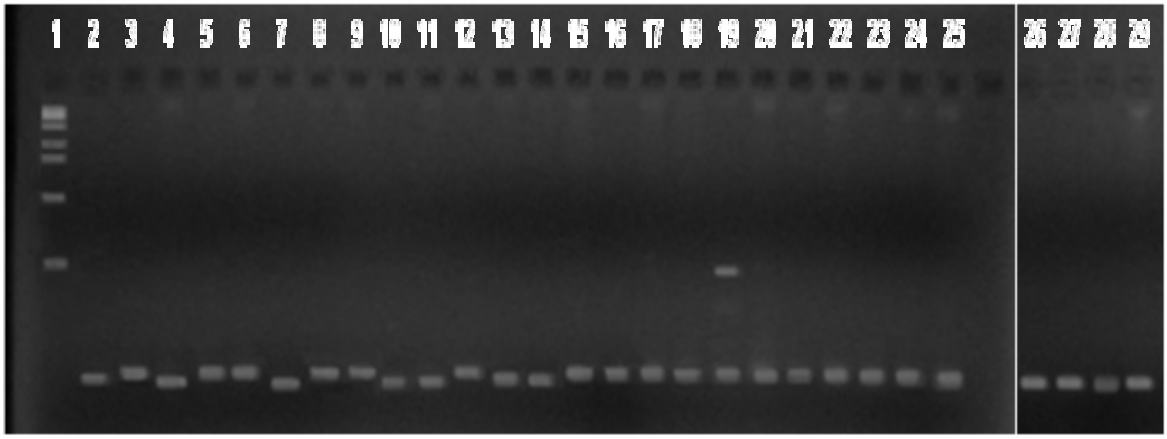

Fig 1. Diagnosis of individual lines carrying blast resistance gene at the locus of RM44

1. Marker φX174 – *Hae* III
2. HG 1
3. IR 24
4. IR29429-B-3-B-2-7
5. IR39357-91-3-2-3
6. IR41996-118-2-1-3
7. IR47686-6-1-1
8. IR48725-B-B-86-2-2
9. IR49517-57-2-3-6-2
10. IR63380-16
11. IR65907-191-1-B
12. LD183-4
13. LST148-250
14. MILYANG 80 (PALGONG BYEO)
15. NANG NHEN
16. OM 2395
17. OM 2496
18. OM 3401
19. OM 3536
20. OM 5239
21. OM 5240
22. OM 5625
23. OM 5637
24. OM 5799
25. OM 5900
26. OM 5981
27. TEP HANH 170 mutant
28. BR729-45-3-2-3-1-1-1
29. RP2167-323-1-2

DNA survey for blast

The diversity of alleles in blast resistance genotypes was measured for four microsatellites (RM21, RM162, RM483 and one STS marker (RG64F-R). One of the blast resistance genes were mapped in rice genome as *Pi-2(t)* on chromosome 6 (Mackill and Bonman 1992), and located 2.8 cM distant from the RFLP marker RG64 (Yu et al 1991); thereby providing an opportunity to marker assisted selection with population. The results that all 40 detected by sequence tagged site marker (RG64F-R) with the same phenotypic selection were used to compare with genotype to check accurate the methods. It exhibited polymorphisms in varieties to demonstrate marker-assisted selection (MAS) reached an accuracy of 100% in SSR markers especially RM483. These methods are enough accurate to apply in practice to select varieties that have blast resistance genes for breeding rice. Te Tep, AS 996, Tep Hanh resistance with race P(OM1). Phenotypic selection was used to compare with genotype to check how accurate the methods were. Polymorphisms in varieties show that MAS reached an accuracy of 100% in STS marker with RG64 and 99.49% in SSR marker with RM21. These methods are enough accurate to apply in practice to select varieties that have blast resistance genes for breeding rice.

The diversity of alleles in blast resistant genotypes was measured for Three microsatellites (RM21, RM162, RM483) and 1 STS marker (RG 64F-R). A similarity matrix based on pair wise

comparisons of pooled data was made using NTSYSpc. The dendrogram showed four major clusters with 32 varieties (Figure 2). AS996 and Te Tep from cluster D together distinct from all others accessions at 60% similarity.

Three allelic conditions of the plants susceptible to the disease were detected: homozygotes for resistance alleles, homozygotes for susceptible alleles and heterozygotes' ones. The detection of individuals of homozygotes for susceptible alleles distinguished from heterozygotes.

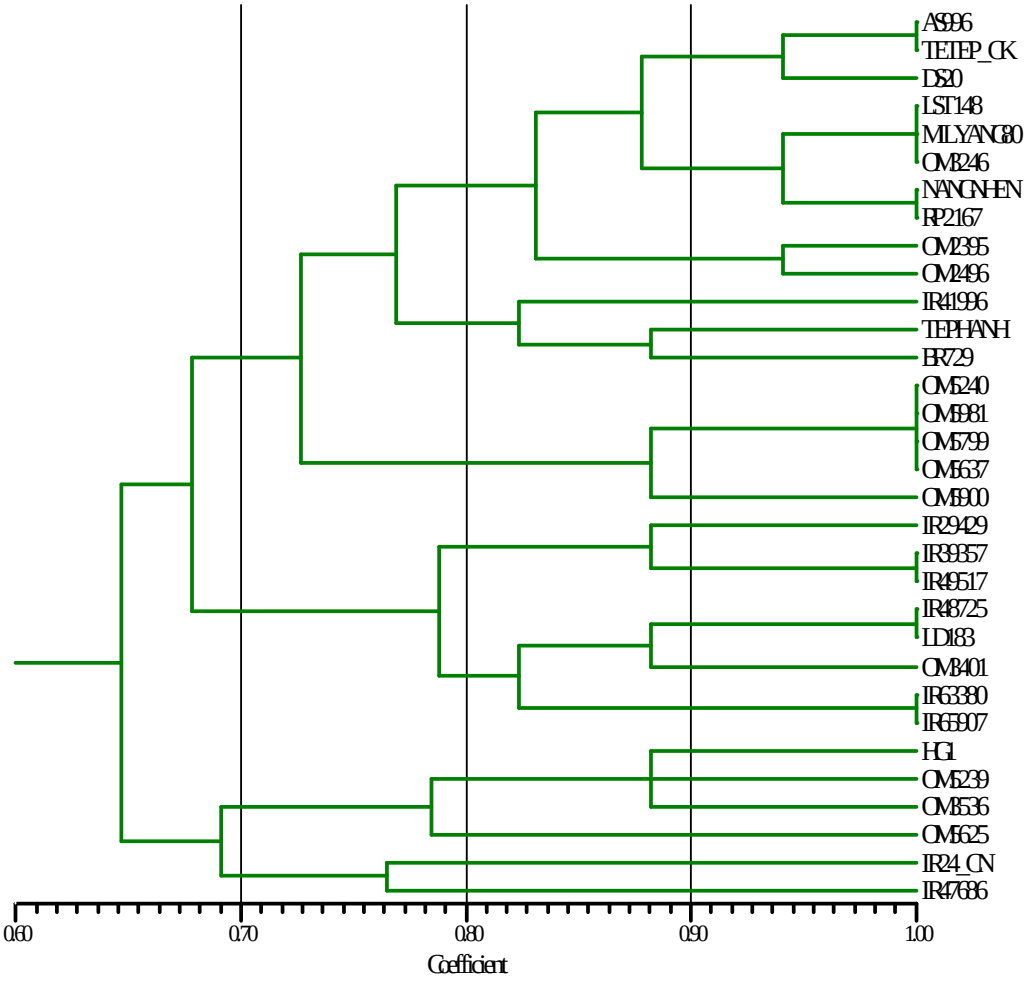

Figure 2. Phylogenetic tree based on SSR and STS profiles

Breeding for bacterial blight (BB)

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is found worldwide and causes substantial damage to rice yield. The rice yield losses of up to 10– 20% were recorded under moderate infection while they were as high as 50% in fields severely affected by bacterial blight (Mew, 1989). Pathogenic specialization had been confirmed in this causal organism (Mew & Vera-Cruz, 1979). Bacterial leaf blight is also an important constraint in successful rice cultivation in Mekong delta. Over the years, some resistance genes for BLB have been identified. Important ones have been pyramided in the high yielding background. Lesion length in these plants varied from 0.00-4.70 cm from difference crosses (table 2).

Table 2: Reaction patterns of F_2 lines to bacterial isolates from Vietnam and IRRI

No.	Designation	Race 1 (PXO280)	Race 2 (PXO61)	Race 3 (PXO341)	Race 4 (PXO339)	Race 11 (CLRRI)
1	IR64	2.97	0.50	0.13	0.30	0.33
2	IR64 x C53	1.23	0.07	0.00	0.00	0.40
3	IR64 x JAS85	1.47	0.10	0.83	0.47	0.00
4	IR64 x OM2514	0.20	0.00	0.13	0.03	0.27
5	IR24	0.00	0.00	0.13	0.80	0.23
6	IR24 x IR64	4.70	0.27	0.17	0.30	0.13
7	IR24 x IR36	0.17	0.33	0.17	0.00	0.50
8	IR36	**1.23**	**0.13**	**0.13**	**0.30**	**0.80**
9	IR36 x IR24	2.40	0.00	2.63	0.10	0.53
10	IR36 x JAS85	0.20	0.00	0.40	0.13	0.00
11	IR36 x OM2514	0.00	0.00	0.13	0.27	0.00
12	C53	0.00	0.00	0.00	0.00	0.00
13	C53 x OM2514	0.30	0.10	0.13	0.87	0.37
14	JAS85	0.13	0.00	0.00	0.00	0.23
15	JAS85 x IR64	0.57	0.00	0.27	0.13	0.13

To evaluate the genetic backgrounds and introgression of resistance genes from donor varieties, the graphical genotypes of the population were constructed using RM13. Three allelic conditions of the plants susceptible to the disease were detected: homozygotes for resistance allele, homozygotes for susceptible allele and heterozygotes.

Table 3: Allelic condition of BB locus in F_2 populations

Numbers	Designation	Allele	Size (bp)	Comparing phenotype from genotype
1	**IR64**	**B**	**150**	**S**
2	IR64 x C53	A,B	500, 150	heterozygotes
3	IR64 x Jasmine85	AB	**500,150**	heterozygotes
4	IR64 x OM2514	A	500	R
5	IR24	B	150	S
6	IR24 x IR64	AB	**500,150**	heterozygotes
7	IR24 x IR36	B	150	S
8	IR36	B	150	S
9	IR36 x IR24	B	150	S
10	IR36 x Jasmine85	AB	**500,150**	heterozygotes
11	**IR36 x OM2514**	B	150	S
12	**C53**	A	500	R
13	**C53 x OM2514**	AB	**500,150**	heterozygotes
14	**Jasmine85**	A	500	R
15	**Jasmine85 x IR64**	AB	**500,150**	heterozygotes

Development of near-isogenic lines (NILs) for bacterial blight gene in rice

Marker-assisted breeding (MAS) has been successfully used by Huang et al. (1997) for pyramiding four resistance genes into IR24 background. DNA marker-assisted selection was employed to select x*a-13 and xa-5 bacterial* blight resistance gene are discussed .Genotypes with both genes were selected from NILs populations involving indica×indica crosses. With the assistance of PCR marker RG136 and RG556, breeding lines carrying *xa-13* were identified from F_2 generation of IR24 / Ba ren cross. Similarly, 15 plants having *xa-5* gene were isolated from and IR24 / Ba ren. Testing was conducted to ensure the selection of homozygous lines for *xa-13* and *xa-5* genes. 258 plants were surveyed for PCR polymorphism in order to facilitate future PCR-based marker assisted transfer of bacterial blight resistance genes *xa-5, xa-13 to* any desired varietals background, which will be useful for selection of parents in breeding programmers. This situation is now possible for several disease resistance genes and for genes related to phenotype and genotype of *xa-5* and *xa-13* (Table 4). In a second case, the marker is genetically associated to the trait of interest for *xa-5*. In this case, xa-*13* lower is the genetic distance between the marker and the gene and more reliable is the application of the marker in MAS because only in few cases the selected marker allele will be separated from the desired trait by a recombination event. Most of the successful applications of MAS discussed below rely on this class of molecular markers. Some examples related to both approaches for the introgression of BC into different genetic backgrounds are discussed.

Table 4: Compare phenotype and genotype of *xa-5 and xa-13* genes of BB

Phenotype	numbers	PCR			Accurracy (%)
		rr	*Rr*	*RR*	
rr *xa-5*	15	14	1	0	93.3
Rr *xa-5*	120	1	118	1	98.0
RR *xa-5*	30	1	2	27	90.0
rr *xa-13*	18	16	2	0	88.8
Rr *xa-13*	50	1	48	1	96.0
RR *xa-13*	25	2	2	21	84.0

Molecular markers are clearly not environmentally regulated and are unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth. With the availability of blast and bacterial blight have of molecular markers and genetic maps, MAS has become possible both for traits governed by major genes as well as for single gene. Usefulness of a given molecular marker is dependent from its capability in revealing polymorphisms in the nucleotide sequence allowing discrimination between different molecular marker alleles. These polymorphisms are revealed by molecular techniques such as microsatellite or simple sequence length polymorphisms (SSR) RM44, RM111, RM 483. A successful application of molecular markers to assist breeding procedures rely on several factors is as

(1) Genetic map with molecular markers linked to the major gene(s);

(2) Tight association between the markers and the major gene(s);

(3) Adequate recombination between the markers associated to the trait(s) of interest and the rest of the population

(4) Possibility of analyzing a large number of individuals in a time and cost effective manner, (5) Success of MAS also depends on the localization of the marker with respect to the target gene

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Nghiên cứu di truyền và chọn tạo giống lúa kháng bệnh đạo ôn và bạc lá

Hiện nay, người ta rất khó quản lý tính kháng bệnh bạc lá lúa. Phát triển tính kháng của cây chủ là biện pháp hiệu quả nhất. Hơn 30 gen kháng bệnh bạc lá lúa đã được phân lập trong đó, Viện Lúa đã tập trung vào gen kháng *xa-5, xa-13, Xa-21 Xa-4.*

Bệnh đạo ôn do nấm *Pyricularia grisea* Cav.*,* là một trong những bệnh hại chính trên cây lúa ở vùng đồng bằng sông Cửu Long. Bệnh có thể lan truyền thành dịch và làm giảm đến 20% năng suất lúa mỗi năm đặc biệt nếu mùa mưa kéo dài thì bệnh càng nặng. Các giống lúa mùa địa phương được coi là vật liệu đa dạng di truyền cung cấp nguồn gen kháng. Do đó, chương trình lai tạo với các giống kháng là mục tiêu quan trọng cho việc cải tiến giống lúa kháng bệnh đạo ôn. Năm tổ hợp lai OM2514/IR64, IR24/OM2514, C53/IR64, C53/OM2514 và IR36/C53 được thực hiện. Nghiên cứu trên 100 giống lúa mùa, đánh dấu RM21, tìm thấy gen *Pi-5(t)* và *Pi-3(t)* ở nhiễm sắc thể số 4. Kết quả so sánh đánh giá kiểu gen và kiểu hình đạt 84,21% và 77,27% tại locus RM21, với hai nòi *P06-6 và Pi-OM 1*. Kết quả này rất có ý nghĩa chính xác cho việc chọn gen kháng đạo ôn. Các giống lúa AS996, Tẻ Tép, Tép hành … kháng với dòng nấm *(P OM-1 và P06-6*). Do đó, các giống trên là nguồn vật liệu rất quý cho việc chồng gen kháng bệnh đạo ôn trong chương trình chọn giống.