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INITIAL MARKER-ASSISTED SELECTION IN RICE BREEDING AT CUU LONG DELTA RICE RESEARCH INSTITUTE

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ABSTRACT

Vietnam has made a big leap in agricultural production with annual increase rate of 4.3%, out of which food production increased by 5.8% per annum. As a result, Vietnam has changed from a country with chronic hunger to one of the world largest rice exporters, and farmers' living standards considerably improved. However, a large proportion of farmers living in coastal salt affected areas of the Mekong and Red River deltas have not benefited much from these developments, owing to the low productivity of these areas caused by persisting abiotic stresses. In addition, population is rapidly increasing and agricultural lands are diminishing due to industrial expansion. Land and water resources in the Vietnam are being exhausted at increasing rate due to soil erosion, land degradation and other causes. Since most agronomic traits composed of more than two genes and, in extreme cases, ten to more genes, breeding strategies to combine them is similar to a "hit or miss" approach, making it extremely difficult to select the most preferable alleles for each of the genes corresponding to a phenotype. To increase and stabilize food productivity and enhance livelihood and food security in salt-affected areas, the following objectives are being targeted: (1) to develop and distribute varieties possessing enhanced tolerance of salt and drought stresses together with accompanying management options, for dissemination, with the potential to double the yield under stress conditions in target areas; (2) to provide NARES with varieties combining tolerance drought and salinity for coastal areas. Three main approaches are being followed to increase and sustain agriculture productivity in Vietnam: (1) increase the area under cultivation by exploring less productive marginal lands, (2) increasing yields through development of high yielding varieties tolerant to prevailing stresses, together with proper management practices, and (3) increasing the values of agricultural products through introduction of more adapted high value crops

Keywords: Amylose content; Aquaculture; Aroma; QTL mapping; Rice; Salinity.

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 diseases as well as abiotic stresses lower rice productivity in the Mekong Delta. Breeding disease resistant rice genotypes using marker assisted selection was reported (Lang et al. 2004a). 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 that 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. With the advent of DNA marker technology, several types of DNA markers are now available to plant breeders and geneticists, helping them to overcome many of the problems faced during conventional breeding. This paper reports on some of the applications of DNA marker technology in our research, for genetic analysis and characterization of various rice accessions, fingerprinting for purity tests, as well as for use in marker assisted backcrossing (MASC) to breed rice varieties and hybrids possessiong particular traits such as specific grain quality and tolerance to particular biotic and abiotic stresses.

Segregation analysis and germplasm/parental surveys

The most important step in segregation analysis is the selection of the appropriate parental lines. The parents should be genetically divergent enough to exhibit sufficient polymorphism. The parents are crossed to produce the segregating population, which could be an F2, backcross, recombinant inbred lines (RILs), or double haploids (DH). For example polymorphism between parents is identified using PCR-based markers. We made 10 crosses (Table1) to evaluate the genetic backgrounds and introgression of new varieties from donor varieties. The graphical genotypes of the population were constructed using RM13 simple sequence repeat (SSR marker). Five allelic conditions of the plants susceptible to the disease were detected: homozygotes for tolerance allele, homozygotes for susceptible allele and heterozygotes.

Table 1: Allelic condition of RM13 locus in F2 populations (with bacterial leaf blight reaction)

No.	Designation	Allele	Size (bp)
1	IR64	B	150
2	IR64 x C53	A, B	300, 150
3	IR64 x Jasmine85	B, E	100-150
4	IR64 x OM2514	A	B
5	IR24	B	150
6	IR24 x IR64	B	150
7	IR24 x IR36	B, C	120-150
8	IR36	C	120
9	IR36 x IR24	C, B	120-150
10	IR36 x Jasmine85	C	120
11	IR36 x OM2514	C, D	120-140
12	C53	A	300
13	C53 x OM2514	A, D	300-140
14	Jasmine85	E	100
15	Jasmine85 x IR64	E, B	100-150

Anther culture shortens the breeding cycle by rapid generation of homozygous lines in a single generation. Anther culture lines from the crosses of C53/Doc Phung and C53/Pokkali, have been developed and genotyped by RM223 (Table 2)

Table 2. Allelic variation of RM 223 locus in anther culture-derived populations (A2)

S. No.	Designation	Allele	Size (bp)
1	IR28	A	160
2	Pokkali	B	140
3	C53/Doc phụng-1	A	160
4	C53/Doc phụng-2	A	160
5	C53/Doc phụng-3	A	160
6	C53/Doc phụng-17	B	140
7	C53/Doc phụng-19	B	140
8	C53/Pokkali-1	A	160
9	C53/Pokkali-2	A	160
10	C53/Pokkali-3	A	160
11	C53/Pokkali-5	B	140
12	C53/Pokkali-11	B	140
13	C53/Pokkali-25	A	160
14	C53/Pokkali-26	A	160
15	C53/Pokkali-27	B	140
16	C53/Pokkali-42	B	140
17	C53/Pokkali-43	B	140
18	C53/Pokkali-44	AB	140-160

DNA markers can also be used for fingerprinting, and this approach has recently been used extensively for determining seed purity such as the example of the genotypes, Ngang thom Cho Dao and Nang Nhen (Table 3).

Table.3. Application of SSR markers for fingerprinting of Nang Nhen variety detected a mixture of 30 lines.

Marker	Numbers of allele	Chromosome	PCR product
RM13	3	5	23
RM223	4	2	23
RM106	3	8	28

Information on the genetic diversity in a crop species is important for selection of parental lines, for construction of populations, for various purposes and also for studying genetic variability within a species. for example from wild rice O. officinalis is shown in Fig. 1

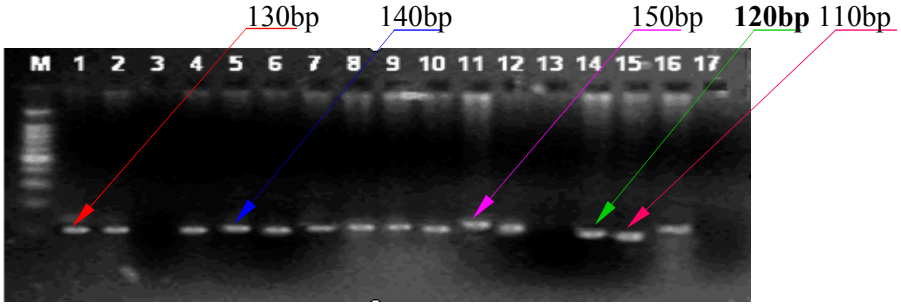

Figure 1. PCR – based DNA characterization of some Oryza officinalis populations at locus RM270.

Table 4. Genotyping of selected lines of O. officinalis using marker RM270.

No.	Designation	Accession	Allele	Size (bp)
M	Marker			
1	O. officinalis		C	130 bp
2	O. officinalis		C	130 bp
3	O. officinalis	193		
4	O. officinalis	195	C	130 bp
5	O. officinalis	196	B	140 bp
6	O. officinalis	216	C	130 bp
7	O. officinalis	236	B	140 bp
8	O. officinalis	235	B	140 bp
9	O. officinalis	231	B	140 bp
10	O. officinalis	234	C	130 bp
11	O. officinalis	230	A	150 bp
12	O. officinalis	233	C	130 bp
13	O. officinalis	229		
14	O. officinalis	232	D	120 bp
15	O. officinalis	228	E	110 bp
16	PTB33		A	150 bp

Understanding germplasm relationships

Understanding and management of the natural variation present within the domestic cultivars and wild relatives of a plant species is very important in the establishment of an efficient program aimed at crop improvement. Exploiting natural variation is very important for several reasons: genetic uniformity in crops is undesirable because it tends to make the crop vulnerable to epidemics and environmental disasters resulting in yield loss. Many wild relatives of crop plants contain genes which confer resistance to biotic stresses such as pests and diseases and tolerance to abiotic stresses such as drought, cold, and salinity (Lang et al, 2005a). When such traits are incorporated into economically important varieties, large yield losses can be avoided. In addition, the breeder also aims at improving certain desired characters such as grain quality and yield for specific end uses. A pre-requisite for improving the overall plant characteristics is an understanding of the structure of the germplasm collection, which in turn will allow a systematic sampling of the germplasm for breeding and conservation purposes.

The genetic diversity and varietal identities of 100 local varieties with bacterial leaf blight resistance was studied. This genetic divesity ranged from 0.24 to 0.60 with an average of 0.40. DNA markers have been used to quantify the genetic diversity and determine phenetic relationships in several rice species. Cluster analysis was useful for studying the relationships among closely related accessions. The diversity of the 100 traditional varieties from Mekong delta was assessed using 34 polymorphic SSR markers and quantitative morphological characters. Molecular diversity analysis revealed genetic diversity among the 100 traditional varieties, which generated five clusters at 0.72 similarity coefficient. Some with the same variety names were classified into different clusters. Though they belong to the same cluster based on morphological markers, molecular analysis showed that they were genetically different. The genetic diversity value ranged from 0.116 to 0.894 with an average of 0.724 (Table 5)

Table 5. Genetic divesity value of 100 local accessions using 34 polymorphic SSR markers.

Cluster	H
I	0.1667
II	0..1171
III	0.6055
IV	0.3633
V	0.8947
Average	0.7242

Both the morphological and the SSR markers were able to classify the rice varieties into these agro-ecological groups, while (principal component analysis) provides a more complete representation of the relationships among major groups. In a "core" collection, the individuals, varieties, or accessions are classified into a limited number of entities based on their degree of similarity and a limited number of genotypes can efficiently represent a much larger group. A "core" collection thus represents most of the diversity in the germplasm collection and allows one to extrapolate conclusions to the entire collection. DNA fingerprinting has been found to extensive applications in assessment of seed purity, resolution of uncertainties, parentage, legal protection of improved varieties and genetic diagnostics.

Construction of genetic linkage maps

DNA markers in plants have been used for the development of detailed linkage maps. In order to efficiently use the innumerable polymorphisms as genetic markers, knowledge of their individual genomic locations is necessary and this information can be obtained by constructing a genetic linkage map. Thus, a genetic linkage map graphically represents the arrangement of the innumerable loci, which include morphological traits, isozymes, as well as DNA markers, along the chromosome. The distance between these loci is expressed in centimorgan (cM) which represents the recombination rates between the loci (1 cM= 10% recombination). Examples of genetic linkage maps constructed in rice at the CLRRI for mapping specific traits are shown in Table 6.

Table 6. Construction of genetic linkage some maijor genes in rice at CLRRI

	Target Trait	Chromosome location of QTLs	Linked markers	Population	Reference
1	QTL for salt stress	12	C560-C747 , R3156- C563, C1454 , C397, R1684	Tenasai 2 / CB	Lang et al . 2001a
2	Salt Tol	8	RM223	IR28/ Doc Phụng	Lang et al. 2001b
3	Salt Tol	1	RM315	IR64/OMCS 2000//OMCS2000; IR64/OM 1706//Om1706	Buu.and.Lang. 2004a
4	Salt Tol	2	OSR1	IR64/OM 1706//Om 1706	Buu and Lang 2004b
5	P deficiency tolerance	12		OM 2395/ AS 996	Lang et al , 2005
6	Drought tolerance	9	RM201 linkage 0,4 cM	OM 1490/WAB880-1-38-18-20-P1-HB	Lang et al., 2007

	Target Trait	Chromosome location of QTLs	Linked markers	Population	Reference
7	Brown plant hopper	12	RM457F-R , linkage at 3.5 cM	PTB33 / TN1	
	Lang et al., 1999				
8	Brown plant hopper Res	12	RM270 , linkage 0.2 cM RM260, 5 cM	IR64/ Hoa Lai	Lang et al., 2005b
9	Aroma	8	RM223, RG28F-R	IR64/ Hoa Lai	Lang et al 2004c
10	Amylose Content	6	RM42, RM276	IR 64/ KhaoDawMali 105	Lang et al 2004d
11	Blast R	6	RG64:1,0cM 3,8 cM - 4,8	OM 1308 / Te tep and Soc Nau / OM997	Lang et al., 2003
12	Grain Protein	7	RM234	BC_2F_2 IR64 / Khao Dawk Mali 105 .	Lang et al., 2005b

Marker Assited Selection (MAS) for major genes

MAS is based on the concept that it is possible to infer the presence of a gene from the presence of a marker that is tightly linked to the gene. If the marker and the gene are located far apart then the possibility, they will be transmitted together to the progeny individuals. They will be reduced due to double crossover recombination events. Hence, a prerequisite to using markers in such selection is that they should be tightly linked to the gene of interest. For this purpose, saturation of specific regions containing the gene of interest on the genetic linkage map is necessary.

Grain quality

This study was undertaken with the view to tag gene(s) controlling grain protein content (GPC) using molecular markers in rice. Genotype IR64 with low protein content (7.5%) was crossed to genotype Nang thom Cho Dao with high protein content (10.62%) and BC3F2 population of 149 lines was derived. The parental genotypes and BC3F2 population were analyzed with RM234. One primer pair for the locus RM234 showed association with protein content. This was further confirmed through selective genotyping. The co-segregation data on the molecular marker and protein content on 149 BC3F2 lines was analysed using single marker linear regression approach. The results showed that the linked QTL accounted for 18.1% of the variation for protein content between the parents. Significant regression suggested linkage between RM234 and a QTL for protein content on chromosome 7. The results showed that this marker-linked QTL accounted for 8.73% of the variation for protein content between the parents. The marker has been located on chromosome arm 7 (Lang et al., 2005b)

Amylose content is one of the important characteristics of grain quality of rice verities. A polymorphic microsatellite sequence closely linked to the Wx gene was reported. .The genotype Hoa lai and Khao Daw Mali 105 with low amylose content (16.8% and 19.2%, respectively) were crossed with IR64, with medium amylose content (24.5%), and 120 BC2F2 lines were derived. These BC populations showed normal distribution for amylose content. One locus associated with amylose content was identified near the Wx gene in both populations ($P < 0.000$, R2= 17.8%, for IR 64/ Hoa Lai and; and $P < 0.0000$, R2 = 19.7% for IR64/KhawdawMali 105). The parental genotypes were analysis with 20 primer pairs for SSR markers on Chromosome 6. This QTL might be useful in marker- assisted breeding for the improvement of rice amylose content.

We also conducted genetic analysis of two traits: amylose content (AC) and gel consistency (GC), as the most important traits for cooking and eating

quality of rice grains. The material used in the analysis included five groups: A0 (duration from 90-95 days) , A1 (duration from 95-100 days), A2 (duration from 105-110-days), abiotic stress tolerant, and genotypes with specific traits such as aroma, together with IR64 ,Khao Daw Mali 105 as checks A microsatellite repeat that is part of the detected with gene amylose with marker RG42 and GC for RM276. Three new varieties with low amylose content such as: OM4900, Hau Giang 2, OM3536 . The higher significantly between phenotype and genotype of two traits .

The aroma or good flavor of cooked rice has been shown to be caused mainly by formaldehydes. The genomic clone RG28, which is tighly linked to frg gene in rice, provides opportunities to perform marker aided selection in rice breeding program. This study was conducted to identify the target gene using SSR markers (RM223) and STS (RG 28FL-RB) markers linked to fgr gene in rice. RG28 can be converted by sequencing into STS and used as marker for PCR amplification of genomic DNA from rice varities differing in their aroma . Genotypes of 16 local varieties and 49 improved varieties were determined by performing progeny testing for fgr. The results indicated an accuracy of close to 100% in identifying aromatic rice plants, which were similar to that using RG28FL-RB. Germplasm survey was conducted for parents selection for use in breeding (Lang et al. 2006) .

Phytic acid, myo-isositol 1, 2, 3, 4, 5, 6 – hexakisphosphate (IP 6) is a major storage compound of phosphorous (P) in plants. Rice grain contains anti-nutritional factors, which reduce the bioavailability of iron and zinc. Phytate has been known to lower the absorption of these cations and other minerals in humans and non-ruminant animals. Induced mutations in the most important cereals have been utilized to reduce the phytate content of rice grains. All efforts to develop mutants with low phytic acid content have been recognized in the development of four rice genotypes from different wild types as OMCS2000 and OM1490. Their rice grains were treated with gamma radiation (20 kr) then screened for high levels of free phosphate in order to identify low phytic acid (LPA) mutants. The LPA phenotype was analyzed in mutants, wild types, 101 improved varieties and 600 landraces. LPA mutants derived from OM1490, OMCS2000 in M3, M4 and M5 showed uniform agronomic traits (Lang et al. 2007)

The advent of marker assisted selection permits rapid identification of individuals that contain genes for low phytic acid. The presence or absence of the associated molecular marker indicates, at an early stage, the presence or absence of the desired target gene. Segregating populations were used to confirm co-segregation between SSR markers and the gene for LPA. Polymorphic marker RM207 detected LPA in OM1490 mutants. Lane 1 (IR64) exhibited band of 200 bp and lane 2 showed Lpa-1 at 220 bp. Lane 3-7 presented high phytic acid allele from the wild type OM1490, lane 8-12 indicated LPA of landraces as Nep than, Nep Hạt to, Nep Ao vang , Nep Thom and Nep Mau Luon. Our findings may help rice breeders to develop low phytic acid genotypes with improved nutritional value to overcome anemia syndrome and meet the demand of biofortification

Biotic stress

Bacterial Blight:

Bacterial leaf blight (BB) caused by Xanthomonas oryzae pv. oryzae (Xoo) is one of the major diseases of rice in the world. In some areas of Asia, it can reduce crop yield by 50% (Khush et al., 1989) or even up to 80% (Singh et al.1977). The disease caused by bacteria, also brings about reduction in the rice production. Plants genes governing highly effective resistance to microbial attack has been identified before, and often, these resistance genes are clustered in specific region of the plant genome (Richter et al., 1995). These resistance genes belong to multigene families, and are closely linked. Resistance to bacterial blight was transferred from wild species O. longistaminata to the cultivated rice variety IR24 generating the introgression line IR-BB21 and the locus Xa-21 was found to confer resistance to all known Xanthomonas oryzae pv. oryzae in India and the Philippines (Khush et al., 1990). About hundred and sixty six local accessions, and 25 parental lines of hybrid rice were screened for leaf blight resistance using 10 international bacterial races in comparison with check varieties. Five local cultivars were identified as resistant of the

bacterial races as IRBB21, 3 cultivars showed resistant reaction as IRBB5 and 58 cultivars were resistant to the race No 4 and No 6 as IRBB13. These cultivars were subsequence genotyped using RG556, RG136 and PTA248 for detecting xa-5, xa-13 and Xa-21 genes. PCR reactions using RG556 and PTA248 failed to detect xa-5 and Xa-21. Marker RG136 was successful in selecting 5 local rice accessions and 3 parental lines of hybrid rice containing xa-13 gene. A susceptible modern cultivar (IR24) and the resistant local genotype (Nang Som) were used for developing a backcross population to transfer recessive xa-13 gene. Bacterial blight resistance gene, xa-13, was not detected in the first BC generation. Screening of 130 plants with IR24 phenotype in BC1F2 population identified 20 plants that were resistant to at least 6 races including race 4 and race 6 (typical resistance phenotype of xa-13 gene). These plants were genotyped with 5 microsatellite markers (RM21, RM114, RM122, RM164, and RM190), of which 2 markers RM21 and RM190, showed polymorphisim with the accuracy of 55% and 50%, respectively. In comparison with BB pathogen reaction, these plants do not have xa-13 gene but their resistance was caused by multiple genes at other loci.

Marker-assisted backcrossing (MABC) 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 xa-13 and xa-5 bacterial blight resistance genes. Genotypes with both genes were selected from NIL populations involving indica x indica crosses. DNA marker-assisted selection was employed to select xa-13, xa-5 and Xa-4 bacterial blight resistance genes. Genotypes with both genes were selected from four BC4F4 populations from IR24/ Base (local Vietnamese variety). With the assistance of PCR-based markers, 60 true breeding lines were identified from CLRRI and 100 BC4F4 populations were also subjected to marker assisted selection for xa-13 locus. Plants were analyzed with the STS marker RG136, which showed polymorphism between IR24 and other parental lines involved in the crosses. PCR analysis was conducted using oligos RG136 in an BC4F4 population segregating for xa-13 locus. OMCS2000 showed banding pattern identical to that of its xa-13 parent, Based on this, these plants were therefore, assumed to carry xa-13 gene in homozygous state. The homozygotes and heterozygotes were scored and goodness of fit was tested. One hundred plants were raised from each selected BC4F4 plant. Sixty (60) lines raised from each selected line were tested against 11 races of bacterial blight pathogen, and all the plants showed resistance to BB pathogen. The resistance reaction was also confirmed using SSR and STS markers for xa-13, xa-5 and Xa-4 resistance genes, and superior plants carrying Xa-5 gene were selected (OM 2517 and OM 5636). Besides, five lines carrying Xa-4 gene (OM 2718, AS996, OM2514 , OMCS2000 and DS2002) were selected. In total, 60 phenotypically superior plants carrying genes for resistance were selected (Phuc and Lang, 2005).

Blast resistance

Blast is caused by Pyricularia grisea Cav., is one of the major fungal diseases of rice in Vietnam. Local varieties have been considered as genetic sources of disease resistance. This study aims at using DNA marker assisted selection to breed varieties that have blast resistance genes such as Pi-2(t), Pi-5(t) and Pi-3(t). STS marker RG64 was used to screen 100 local varieties for the presence of Pi-2(t) in chromosome 6, and SSR marker RM21 was used for Pi-5(t) and Pi-3(t) genes in chromosome 4. Phenotypic evaluation was used to compare selected lines with checks to evaluate the accuracy of genotypic selection using these markers, and the data showed that MAS has an accuracy of 100% for the STS marker RG64 and 99.49% for SSR marker RM21. These markers, are therefore sufficiently accurate for use in breeding to select breeding lines that have blast resistance genes. The local genotypes Nang Huong, Lem Bui, Soi Da, Nang Tra, Nang Tra Ran Doc, Soc Nau, and Te Tep have 3 blast resistance genes Pi-2(t), Pi-5(t), and Pi-3(t), and are considered as valuable material for pyramiding resistance genes to develop varieties with durable resistance.

Development of blast resistant rice varieties

The resistance genes in CLRRI rice varieties were examined based on reaction patterns of isolates of blast Pyricularia grisea Sacc., from CLRRI. To

confirm the genes, genetic analyses were carried out using BC1F2 progenies derived from crosses of IRRI varieties with IR24 and IR36 . This study demonstrated the utility of differential system in elucidating the genetic constitution of CLRRI and IRRI varieties for blast resistance (Table 8). A backcross population consisting of 118 BC2F2 lines derived from IR24 / OM2514 was analysed for blast resistance genes and genotyped with 14 simple sequence repeat (SSR) markers. One SSR marker (RM21) was significantly associated with blast resistance in rice (P=0.01). These markers accounted for phenotypic variation ranging from 9.6% to 20.5% and contributed to 66% of the total variation of percentage diseased leaf area (DLA) observed under natural infection. To evaluate the genetic backgrounds and introgression of resistance genes from donor varieties, the graphical genotypes of the population were constructed using 3 SSR markers, RM44, RM111, RM483 and marker φX174 – HaeIII (Fig 2). These materials will further be used for breeding.

Table 7. Reaction pattern of F2 lines from IR24/OM2514 and IR36/OM2514 to blast isolates from CLRRI (OMP -1)

Designation	Score reaction 0	Score reaction 1	Score reaction 3	Score reaction 5	Score reaction 7	Score reaction 9
IR24						30
OM2514		30				
F1	50	32	27	44	27	75
BC1F2	32	45	22	44	27	75
IR36 / OM2514						
IR36						10
OM2514		30				
F1	50					
BC1F2	12	40	34	27	50	25

A set of 28 differential varieties is a useful tool to identify blast resistance genes in rice and to characterize the new varieties. The reactions of 28 lines to the blast isolates from CLRRI were confirmed using markers RM44, RM111, RM483 together with marker φX174 – Hae III .

Introgression of a resistance gene for brown plant hopper (BPH)

Several wild species of rice with high degree of resistance to pests have been identified at IRRI. Similarly, O. rufipogon, a wild rice is tolerant to acid sulfate soils that occurs in Dong thap Muoi, Vietnam. The wild species thus offer great potential to transfer genes for tolerance to biotic and abiotic stresses into rice cultivars. CLRRI has generated a series of hybrids, and introgression lines from the crosses of elite breeding lines of rice with several wild species. Genes for resistance to brown plant hopper (BPH), bacterial leaf blight and blast have been transferred from several wild relatives into cultivated rice. The BPH resistant line from O. sativa / O. officinalis have been released as commercial variety for cultivation in Mekong Delta such as AS 996. Some of the genes introgressed from wild species have been tagged with molecular markers. IRRI and CLRRI have strong on-going collaboration on the evaluation and utilization of wide cross progenies. Under the Rockefeller Foundation (RF) support, tagging of BPH resistance loci was conducted with microsatellite markers at Texas A&M University from Oryza officinalis / IR50 (Buu et al., 2005). It showed that the genes for BPH resistance (biotype 4) are linked with RM18 on chromosome 7 at a distance of 1.3 cM, and RM168 on chromosome 3 at a distance of 1.9 cM.

Backcross progenies from crosses with IR64 / O. rufipogon and IR64 / O. officinalis were evaluated for BPH resistance. The results indicated that O. officinalis was a good donor resistant to BPH (Table 8)

Table 8. Reaction patterns of BC2F1 lines to BPH from Vietnam

		Bph		RGSV		RRSV	??
Numbers	Designation	Numbers of suceptible plants	Numbers of resistant plants	Numbers of suceptible plants	Numbers R f (medium) Plants	Numbers for R resistance)	
1	IR64/ *O. Rufipogon*	56	170	26	226	0	226
2	IR 64/ Nang Thom Cho Dao	356	12	15	353	10	358
3	IR 64/ *O. officinalis*	**30**	**91**	**0**	**121**	**0**	**121**

R: resistance

Brown plant hopper causes direct damage by sucking plant sap, and it also tranmits several viral diseases such as rice grassy stunt (RGSV; Rivera et al 1966) and ragged stunt (RRSV; Ling et al., 1978). About 121 lines derived from the cross IR64 / O. officinalis were evaluated. It indicated that all lines were resistant to RGSV and RRSV while lines derived from IR64 / Nang Thom Cho Dao and IR64 /O. rufipogon were less resistant.

The survey revealed three markers RM270 linked to target regions on chromosome 12 that contained genes for BPH resistance. O. officinalis had large effects on BPH resistance. The five loci independently acted of each other in determining the resistance. However, the record did not detect polymorphism for Oryza rufipogon.

Abiotic stress

Environmental stresses such as drought, salinity, submergence, and phosphorus deficiency are major factors limiting plant productivity. Plants have developed different physiological and biochemical strategies to adapt or tolerate to these stress conditions in response to various environments. Tolerances of plants to environmental stresses sometimes involve the accumulation of compatible low-molecular weight osmolytes such as sugar alcohols, special amino acids, and glycine betaine as an adaptation mechanism, especially under water stress.

Drought tolerance

In most rice growing areas, yield reduction due to drought have been observed. To overcome this problem, A marker-assisted backcrossing (MABC) breeding programme was initiated to improve the root morphological traits, and thereby, drought tolerance of the Vietnam upland rice varieties. The recurrent parent in the advanced bacrossing had not previously been used for quantitative trait locus (QTL) mapping. The donor parents used were WAB 880-1-38-18-20-P1, IR65195-3B-2-2-2-2 and WAB881 SG9 from IRRI. These lines were crossed with OM1490 and OM4495 (indica varieties). A linkage map was constructed with SSR markers spanning 260.4 cM along chromosome 9 with average interval of 16.13 cM and used for QTL mapping with MapMarker/ QTL. LOD score of 3.0 was used as the threshold to identify the putative QTLs. Both parents contain favorable QTLs affecting this trait, suggesting the likelihood of recovering transgressive segregants. Phenotypic and genotypic evaluations using 20 markers on a total of 229 lines was completed. BC2F2 lines were evaluated for root length (RL), spikelet fertility (SF), drought recovery score (DRS) and yield (Y) at CLRRI. A target segment on chromosome 9 (RM201) signifcantly increased root length and DT under drought stress treatments, confirming that this root length QTL from OM1490 / WAB 880-1-38-18-20-P1; OM1490/WAB881 SG9, OM4495/IR65195-3B-2-2-2-2. The data suggest that drought tolerance for yield components is largely associated with genetic and physiological factors independent from those determining the traits per se. The implications of these results for developing an

efficient strategy of marker-assisted selection for drought tolerances are discussed.

Phosphorus deficiency tolerance

A molecular map was constructed according to published microsatellites data from Cornell University and from Japan. About 116 microsetellite markers were assigned to linkage groups using MapMarker. Although there are a few gaps of more than 50 cM, the linkage map had a total map length of 2,905.50 cM. The average interval size was 23.05 cM, the smallest size was in chromosome 12 and chromosome 9 (12.50cM) and the largest size was in chromosome 3. There are a few gaps larger than 50 cM. It indicated that the genetically related parents cause the low turn of polymorphism for microsatellite markers.

A mapping population of 225 RIL lines (recombinant inbred lines) was derived from a cross between OM2395 / AS996 by single seed descent method. This population was used to detect quantitative trait loci (QTL) associated with P-deficiency tolerance. From the random sample and SMA (single maker analysis), F-tests were significant, indicating markers associated with P-deficiency tolerance. The results showed that individual putative QTLs explained an average of phenotypic variation ranging from 11.01% to 11.67%. RM247 and RM235 showed the highest F-value ($P<0.001$) and therefore are most likely to be linked to the P-deficiency tolerance trait. Five QTLs were detected in chromosome 1, 2, 5, 9 and 12. Composite interval mapping was implemented using QTL mappmarker Software, with a threshold of LOD>3.0. Three putative QTLs were detected with percentage of variance explaining between 11.2% - 9.13% of the variation in root length and root dry weight, respectively.

Salinity tolerance

Salinity is one of the major problems in agriculture, limiting crop growth and production in many parts of the world. We investigated the genetic basis of salinity tolerance and evaluated improved varieties using microsatellite markers. Phenotypes were evaluated by visual scores of salt injury at vegetative and reproductive stages under salt stress of EC = 12 dS/m in phytotron. To examine the power of the identified SSR marker in predicting the phenotype of the salt locus, we determined the genotypes of 93 improved varieties at RM223 locus. The results indicated an accuracy of more than 95 % in identifying the tolerant plants, which was similar to that using RM223.

The results of the germplasm survey using genotypes such as OM5900, OM5930, OM6036, OM6037, OM6043, OM6041, OM6042, AS996, OM3729, OM4675 will be useful for the selection of parents in breeding programs aimed at transferring these genes from one background to another and use in marker assisted selection. These newly developed varieties gave significantly higher yields over three years of testing and results indicated its superiority over the control varieties Pokkali and Doc Do. Results of evaluation of these genotypes at 12 locations showed higher yields under field conditions, confirming their superiority in saline areas.

Method which has been used to identify markers tightly linked to a gene of interest makes use of more population (F2, BC,….) which differ in the presence or absence of the target gene and a small region flanking the target gene. If the source of the gene and the recurrent parent are sufficiently divergent, markers will reveal polymorphisms between the population and the recurrent parents. In this strategy, markers need not be mapped by the usual segregation analysis in order to be localized near the target gene. A breakthrough has been achieved in breeding, some promising combination with high yield from 5-7 ton/ha (Table 9)

Table 9. Some examples of lines successfully developed using MAS.

No	Designation	Parentage	Marker	Trait	Released Year
1	OM4495	IR64/OM1706//IR64	RM42	Good grain quality	2005
2	OM4498	IR64/CS2000//IR64	RM223	Good grain quality, salt and BPH tolerant and high yield	2007
3	OM4900	C53/Jasmine 85// Jasmine 85	RG28, RM264, RM42	Good grain quality, aroma and high yield	promising
4	OM5239	IR64/OM2395	RM42	high yield	2007
5	OM5240	IR64/BusoK	RM42	high yield	promising
6	OM6055	OMCS 2000/D43	RM223	high yield, short days	promising
7	OM5636	D26/IR68//IR68	RM241	P def. tolerance	promising
8	OM5635	D20/IR68//IR68	RM42	high yield, short days	
9	OM5634	D19/IR68//IR68	waxy	high yield, short days	
10	OM5900	AS996/IR50404	RM315	Salt tolerance	
11	OM5936	OM1490-55/C53	RM223	high yield, short days	promising
12	OM6162	C50/Jasmine 85	RG28, RM42, RM270	Good grain quality high yield, short days	promising
13	OM6073	C3/D3//C3	RM270	high yield, short days	promising

CONCLUSION

Molecular markers reveal polymorphisms at the DNA level. With the rapid progress in technological innovations, a lot of new molecular resources and tools such as molecular markers for precise genetic mapping, and, high-quality genome sequence for comprehensive molecular analysis of genome structure and function, have already become available for rice. Diversity within the genomic components is very important for finding new alleles for breeding novel varieties and for understanding the molecular bases of the variation in different traits. One of the main objectives for plant breeders is to improve existing cultivars, which are deficient in one or more traits by crossing such cultivars with lines that possess the desired trait. With the advent of DNA marker technology, several types of DNA markers and molecular breeding strategies are now available to plant breeders and geneticists, helping them to overcome many of the problems faced during conventional breeding. The applications of DNA marker technology in the genetic analysis and improvement of crop plants is now more feasible. The impact can be observed not only by the great progress in the field of molecular biology, but also in many achievements in other related fields of science. Currently, there is a greater possibility to boost rice production to meet the increasing demand for food and overcoming the problem of malnutrition than ever before.

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Bước đầu thực hiện MAS trong chọn tạo giống lúa tại CLRRI

Chon tạo giống lúa nhờ chỉ thị phân tử được thực hiện từ năm 1996 cho đến 2009 tại Viện Lúa ĐBSCL đã cho những kết quả bước đầu như sau:

- Chọn giống lúa kháng rầy nâu từ nguồn gen của lúa hoang được áp dụng bởi marker: RM457F-R; RM270; RM260
- Chọn giống lúa kháng đạo ôn từ nguồn gen của lúa bản địa được áp dụng bởi marker: RM21; RG64
- Chọn giống lúa kháng bạc lá từ nguồn gen của lúa bản địa, lúa hoang được áp dụng bởi marker: RG136
- Chọn giống lúa chống chịu thiếu P từ nguồn gen của lúa bản địa được áp dụng bởi marker: RM 247, RM 235.
- Chọn giống lúa chống chịu thiếu mặn từ nguồn gen của lúa bản địa được áp dụng bởi marker: C560-C747, R3156- C563, C1454, C397, R1684, RM223, RM315, OSR1
- Chọn giống lúa chống chịu khô hạn từ nguồn gen của lúa bản địa được áp dụng bởi marker: RM201
- Chọn giống lúa có hàm lượng amylose thấp đến trung bình từ nguồn gen của lúa bản địa được áp dụng bởi marker: RM42, RM276
- Chọn giống lúa có hàm lượng protein trong hạt cao từ nguồn gen của lúa bản địa được áp dụng bởi marker: RM234
- Chọn giống lúa có mùi thơm từ nguồn gen lúa bản địa được áp dụng bởi marker: RM223, RG28F-R