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Full Length Research Paper

Monogenic lines resistance to blast disease in rice (*Oryza sativa* l.) in Vietnam

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Blast, caused by *Pyricularia grisea* Cav., is one of the major fungal diseases infected rice (*Oryza sativa* L.) in Vietnam. This disease occurs in Vietnam which causes the yield loss of up to 20% particularly in a year with long wet season. Local varieties have been considered as genetic sources of disease resistance among crops. The breeding program was aimed at improvement of blast resistant varieties. Six crosses of plants, OM 24/IR 64, IR 24/OM 2514, C 53/IR 64, C53/OM 2514, OM 1308/TeTep and IR 36/C 53 were obtained. Four crosses were mapped using molecular marker. The resistance genes are inherited dominant and located on chromosomes 6, 8 and 11. A simple sequence repeat (SSR) marker (RM 483) was used to detect 100 local varieties to find resistance with some race at Mekong delta. Phenotypic selection was used to compare with genotype in order to check how accurate the polymorphisms in varieties show that marker assisted selection (MAS) reached an accuracy of 100% in SSR marker with RM 483. These methods can be applied in practice to select varieties that have blast resistance genes for breeding rice, because of their high precision levels. Polymorphisms also show that MAS reached an accuracy of 100% in sequence tagged site (STS) marker with RG64 and 99.49% in SSR marker with RM21. Several blast resistant rice varieties [P(OM 1), OMP 2, OM P4, OMP 5, and OMP 6] have been reported by many researchers. These are considered as valuable material for pyramiding resistance genes to create durable resistant varieties.

Keys words: *Oryza sativa,* blast resistance, polymerase chain reaction, simple sequence repeat, sequence tagged site (STS) marker.

INTRODUCTION

Rice blast disease, caused by *Pyricularia grisea* Cav., is one of the most devastating diseases of rice (Oush, 1985). The genetic constitution for blast resistance of rice varieties bred at the VietNam is poorly characterized. The genetic characterization of resistance in varieties was undertaken following a differential system based on the gene for gene relationship between rice resistance genes and avirulence genes in the blast pathogen. A number of genes conferring resistance to blast have been identified and utilized in rice breeding programs. 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. The most economical protection from the diseases is planting resistant varieties. The breeding of resistant varieties is an effective approach to eliminate the use of pesticides and minimize crop losses due to this disease. However, resistant varieties often break down within 3 to 5 years after they are released for cultivation. To breed rice varieties with more durable blast resistance, multiple resistance genes must be incorporated into individual varieties. The availability of molecular maps in rice has opened new avenues to tag genes governing agronomic traits with molecular markers. This has led to major advances in maker assisted selection and pyramiding of useful genes.

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Abbreviations: SSR; Simple sequence repeat, **MAS;** marker assisted selection, **STS;** sequence tagged site, **PCR;** polymerase chain reaction, **RFLP;** restriction fragment length polymorphism, **EDTA;** ethylenediamine tetra-acetic acid, **SDS;** sodium dodecyl sulfate, **TE;** tris HCl-EDTA buffer, **TAE;** tris acetate- EDTA buffer, **LOD;** limit of detection, **CLRRI;** Cuulong delta rice research institute, **cM;** centi-Morgan.

Table 1. Progenies studied for genetic analysis of resistance to Blast.

Cross	Resistance parent	Resistance to	Susceptible	No. of progeny plants
IR 24 / IR 64	IR 64	OM P1	IR 24	109 F2
IR 24/OM 2514	OM 2514	OMP 1	IR 24	46F2
C 53/ IR 64	IR 64	OM P2	-	61F2
C 53/ OM 2514	OM 2514	OMP 2	-	101F2
IR 36/ C 53	C 53	OMP 1 and OMP 2	IR 36	48F2
Om1308/Te Tep	Te Tep	OM P1, OMP 2,	OM 1308	92 F2 ,56 Bc1F2

Some markers used for blast resistance – *Pil, Pi 2(t), Pita, Pit, Pi7(t), Pi9(t), Pi1(t) and Pib,* have available molecular maps (Khush and Brar, 2004). In the present article, we report the development of molecular markers for selection of resistant mono race, a goal of many rice breeding programs.

MATERIAL AND METHODS

Collection of diseased rice samples

Blast leaves with typical lesions were collected from farmers field at irrigated regions in 6 provinces of Mekong Delta (MD) area (Can Tho = race OMP 01), AnGiang=OMP 02, Long An = OMP03, Dong Thap = OMP4, Hau Giang= OMP 5 and Vinh Long =OMP 6) from season Dong Xuan 2005. All monocultures were maintained in medium for long term storage at Genebank at CLRRI.

Genetic material and disease evaluation

Six crosses involving 3 different resistant varieties (Table 1) out of 100 local and 32 improved varieties using this experiment. Te Tep and IR 24 for checked the 138F2 individuals of each cross were grown in a plastic tray under standard green house conditions for phenotypic disease scoring. The parental resistant and susceptible cultivars were also included as controls. 21 day old seedlings were inoculated with blast isolates *OM P1, OMP 2, OMP 3, OMP 4, OMP 5, and OM P6* (from VietNam). The rice seedlings were sprayed with 50 ml of blast inoculum suspension per tray (5×10^4 condia/ml) and incubated for 24 h in a controlled temperature of 25°C. The seedlings were then transferred to a temperature and humidity controlled chamber. Plants were observed for blast disease symptoms for 1 week after inoculation and scored for resistance and susceptibility when the typical blast lesions developed on the susceptible parental cultivar IR 24. This phenotypic evaluated was repeated 3 times.

Rapid isolation of rice DNA

Suitable rice DNA for PCR analysis was isolated using the method of Lang (2002). 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, 300 mM NaCl and 1% SDS). Grinding was done until the buffer turned green which is an sindication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of the extraction buffer was added and mixed into the well using a pipette. Then 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 50 µl of TE buffer (10 mM Tris- HCl pH 8.0, 1 mM EDTA pH 8.0). An aliquot of 1µl was used for the PCR analysis and thereafter, DNA quality and quantity were spectrophotometrically determined.

PCR amplification

The genomic DNA from both of the $148F_2$ plants and the parents were subjected to PCR amplification using the synthesized primers. The PCR buffer consisted of: 10 mM Tris pH 8.4, 50 mM KCl,1.8 mM $MgCl_2$, 0.01 mg/ml gelatin and *Taq* [5 units of *Taq* polymerase in a volume of 25 µl]. Template DNA were initially denatured at 94°C for 5 min, followed by 30 cycles of PCR amplification under the following parameters: 1 min denaturation at 94°C, 1 min primer annealing at 55°C and 2 min primer extension at 72°C. Final 5 min incubation at 72°C was allowed for completion of primer extension on a 480-thermalcycler. The amplified products were electrophoretically resolved on 1% agarose gel using 1X TAE buffer.

Linkage analysis

Linkage analysis for the segregating polymorphic markers and the blast resistance gene was conducted with MAPMAKER V.2.0 (Lander et. al., 1987) for each of the individual populations. All map distances (centi-Morgans, cM) are reported in Kosambi units (Kosambi, 1994) and critical LOD score thresholds of 3.0 and 0.05 were used for determining linkage groups and for the calculation of map distance in JOINMAPV.14. The segregation ratios of individual markers were calculated with the software program QGENE V.2.0 (Nelson 1994), and skewing was indicated when the ratio deviated significantly from the expected.

RESULTS AND DISCUSSION

Resistance tests and genetic mapping

Analysis of the F2 population

DNA genome from the F2 population, two of the cross between IR24/IR 64 (109 F2 plants), IR 24/ Om 2514 (42 plants of F2) were assessed through PCR amplification using 13 primers (RM 225, RM 314, RM 111, RM 253, RM 50, RM 276, RM 136, RM 83, RM 541, RM 162, RM 276, RM 343 and RM 30) on chromosome 6. Four (4) fragments were resolved on agarose gels and the banding patterns were scored with reference to those of the parents. The banding pattern of the F2 individuals could be classified into homozygote for the IR 24 type marker size 210 fragment, homozygote for Ir64 type mar-

Table 2. Segregation ratios for Blast resistance.

Cross	Segregation R : S	Chi- square test (3:1)	Infection rate (%)
IR 24 / IR 64	79:30	0.25	95
IR 24/ OM 2514	35:11	0.00	98
C53/ IR64	46:15	0.003	92
C53/ OM 2514	84:17	2.56	92
IR 36/ C53	36:12	0.00	95
OM 1308/ TeTep	83:16	2.56	92

ker 200 bp fragment with primers RM 162.

In the first step, the resistant reaction of 148F2 plants against blast was monitored. In all crosses the segregation displayed a 3:1 ratio (resistance: susceptible) as expected from previous experiments which indicated the presence of single dominance genes for all the resistant parents. Infection rates varied from 92-98%; the progeny of resistant F2 plants was tested in plants in F3 generation. Segregation ratios for resistance to blast were summarized in Table 2.

According to the resistance data from the F2 generation phenotypic pools, mapped molecular markers were used for simple sequence repeat (SSR) in chromosome until a region was identified in each cross where the markers showed a clear differentiation between resistance and susceptible (Figure 1).

Gene to race OMP 1

The co-dominant SSR 16 markers have been mapped in both two populations at a distance of 3.9 cM with cross IR 24/IR 64 (102 F2) and 3 cM cross IR 24/OM 2514 (46F 2), respectively (Figure 1).

Gene to race OM P2

The resistance gene has been localized on chromosome 11 with marker RM21 in population F2 from C 53/OM 2514 at distance of 7.7 cM (Figure 2).

Gene to race OM P 3

The population F2 from the cross IR 36/ C53 resistant race OM P3 polymorphism with 11 markers and was located on chromosome 8 with marker RM 483 at a distance of 0.7 cM (Figure 2) .

Molecular marker for marker assisted breeding strategies

DNA markers that are useful for marker-assisted selection inbreeding programs for the introgression of resistance genes from donor lines into adapted germplasm have been identified. SSR markers are linked to blast races OM P1, OMP 2, and OMP 3 in VietNam in the coupling phase and therefore are suitable for selecting homozygous resistance as well as heterozygous susceptible plants, thereby avoiding the necessity of two backcrossing steps which would be indispensable to identify carriers of dominance genes in classical breeding programs. Similarly, SSR marker RM 21 is useful for the identification of lines carrying the blast, and this is in correspondence with (C53 / OM 2514) in the homozygous or heterozygous state. On the other hand, RM 162 is linked to OMP 1 in repulsion and hence allows selection of homozygous resistant lines which are characterized by the lack of the SSR fragment. In this case, however, samples that failed to amplify can not be differentiated from resistant plants. Therefore, stringent controls have to be included into the experimental design. Some of the RG 64 markers on chromosome 6 were linked with population on F2 from OM 1308/ Te Tep (Lang et. al., 2001). Population from OM 1038/ Te Tep were evaluated and compared to identify a resistant plant with race OM P4, based on the genotype linked with RG 64 marker that indicated a matching of up to 96:49. There was accuracy in identifying individuals carrying the resistance gene by selecting from 92 lines F2: 37/92 reaction (R); 43/92 reaction (S) score and 12 /92 reaction (MR) score heterozygote. In this population, a series of micro-satellite markers in close linkage to resistance genes were effective against blast. Molecular markers with SSR primers (RM 162, RM 541) were detected in F2 (92 plants) and BC1 F2 population (56 plants) from Om 1308/TeTep .There may be differences regarding the mechanisms of resistance between individual resistance genes against the blast complex. This can be deduced from the observation that some resistant lines, such as Te Tep, were resistant in the field under natural infection conditions but became infected in the greenhouse upon mechanical inoculation of the leaves.

In order to further characterize the molecular mechanisms of resistance to pathogens, high-density molecular linkage maps can be a starting point for cloning of the corresponding genes via a map-based cloning approach.

Closely linked DNA markers have been identified for the Blast resistance genes. A single marker, RM 162, was used to identify blast genes in a segregating popula-

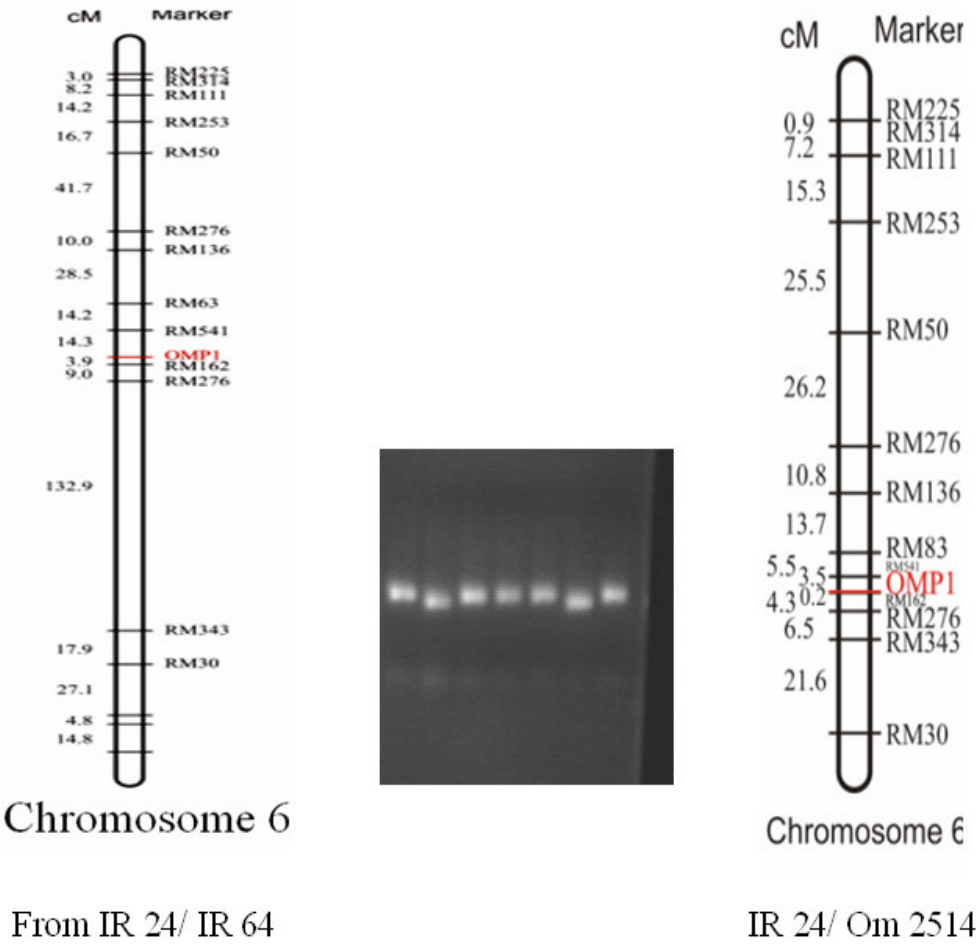

Figure 1. Molecular linkage maps of chromosomal regions harbouring the resistance gene *OMP-1*.

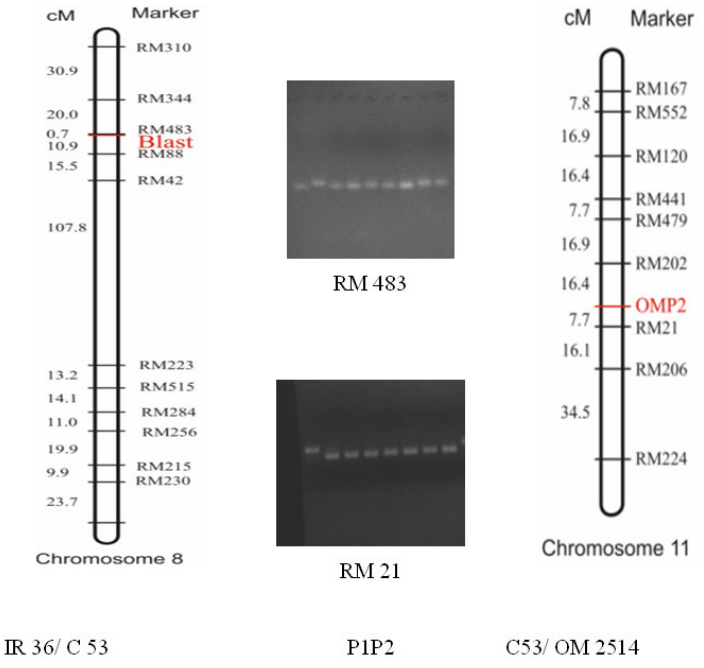

Figure 2. Molecular linkage maps of chromosomal regions harbouring the resistance gene *OMP-2 and OMP 1 + OMP*

Table 3. Presiction of genotypes based on RM 162 at the blast (*OM P 1*) and *OMP 4* lucus in F3 population of OM 1308/ Te Tep.

Phenotype	No. Plants	Genotype		Accuracy (%)
		R	S	
OMP 4				
S	52	1	51	98.07
R	19	16	3	84.21
OMP1				
S	49	2	47	95.91
R	22	17	5	77.27

tion of 71 F3 individuals (OM 1308/ Te Tep) (Table 3).

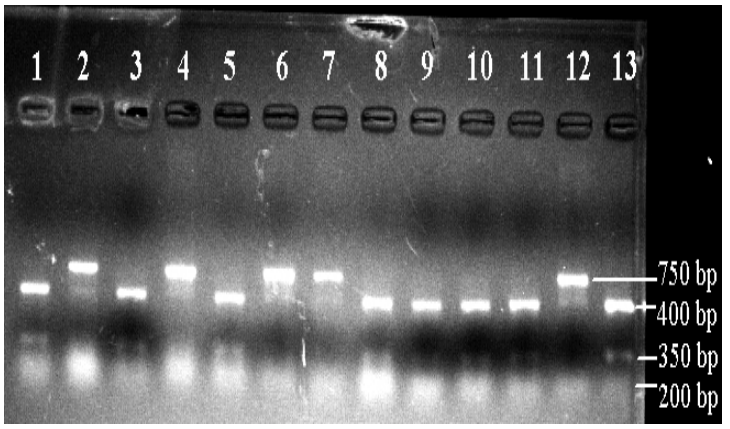

Figure 3a. PCR production of the local varieties based on the banding pattern of the RG64 locus digested with *Hea*III, gel agarose 1.5%, TAE 1X. M: marker: kb ladder: lines : 1: Te Tep , 2: IR64, 3: Soc Nau , 4: Tiêu Đôi, 5: Tai Nguyen, 6: Nang Quot, 7: Nep Mu U, 8: Lem Bụi, 9: Soi Da, 10: Nang Tra, 11: Nmang Tra Ran Doc, 12: Nang Huong, 13: Nang Thom Cho Dao

Germplasm analysis

The genetic diversity of a population depends upon number and frequency of alleles (allelic composition). Microsatellite markers have extensively been used in molecular characterization, genetic diversity studies and management of genetic resources in seed gene banks. Blast resistance genotypes were measured for four microsatellites (RM 21, RM 162, and RM 483) and one STS marker (RG 64F-R). One of the blast resistance genes mapped in rice *Pi-2(t)* were mapped on chromosome 6 (Mackill and Bonman, 1992), and located 2.8 cM distance from the RFLP marker, RG 64 (Yu et. al., 1991) to check the closeness between the genotype and phenotype and confirm these markers. Several DNA markers have been identified which are useful for marker assisted selection in breeding program for the introgression of resistance genes from donor lines into adapted germplasm. 100 local varieties, with 57 accessions, have resistance and 43 susceptible with 6 races from CLRRI (OMP 1, OMP 2, OMP 3, OMP 4, OMP 5, and OMP 6).

Table 4 shows the reaction of phenotype and genotype for the OM P4, OM P5, OM P6, OM P1, OM P2 and OM P3 blast resistance loci. The genotype was compared with the phenotype: + 57/100 reactions (R) score 57% and + 43/100 reaction (S) scores 43%. The results of all 105 lines detected by sequence tagged site marker (RG 64F-R) with the same phenotypic selection were used to compare with the genotype to check for 60% accuracy with RG 64 and race OM P4. At the same time, the phenotypic selection and comparison with the genotype was also used to ascertain how accurate the methods were. Polymorphisms in varieties showed that MAS reached an accuracy of 99.49% in SSR marker with RM21. These methods are, therefore, accurate enough to be applied in practice to select varieties that have blast resistance genes for breeding rice. Several blast resistant rice varieties [P(OM 1), OMP 2, OM P4, OMP 5, and OMP 6] have been reported by many researchers (Nông et. al., 2004). These are considered as valuable materials for pyramiding resistance genes to create durably resistant varieties (Table 4).

The diversity of alleles in blast resistant genotypes was also measured for 32 improved varieties using three micro-satellites and 1 STS marker (RM 21, RM 162, RM 483, and RG 64F-R). A Similarity matrix based on pair wise comparisons of pooled data was made using NTSYSpc. The dendrogram showed 4 major clusters with 32 varieties (Figures 3a and 3b and Table 5). AS 996 and Te Tep group (D) together are distinct from all other accessions at 60% similarity. Three allelic conditions of the plants susceptible to the disease were detected: Homozygotes for resistance allele, homozygotes for susceptible allele and heterozygotes.

DISCUSSION

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 blast in the field, which has been previously mapped with RM162 We found a flanking marker, RM162, and used both the flanking markers, RM 162 and RG 64, to perform marker-aided selection. Selection accuracy was 100% in identifying homozygous blast plants from a segregating F2 population. These results demonstrate 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 further crosses. PCR methodology provides a more effective means of performing marker-aided selection than screening by PCR based

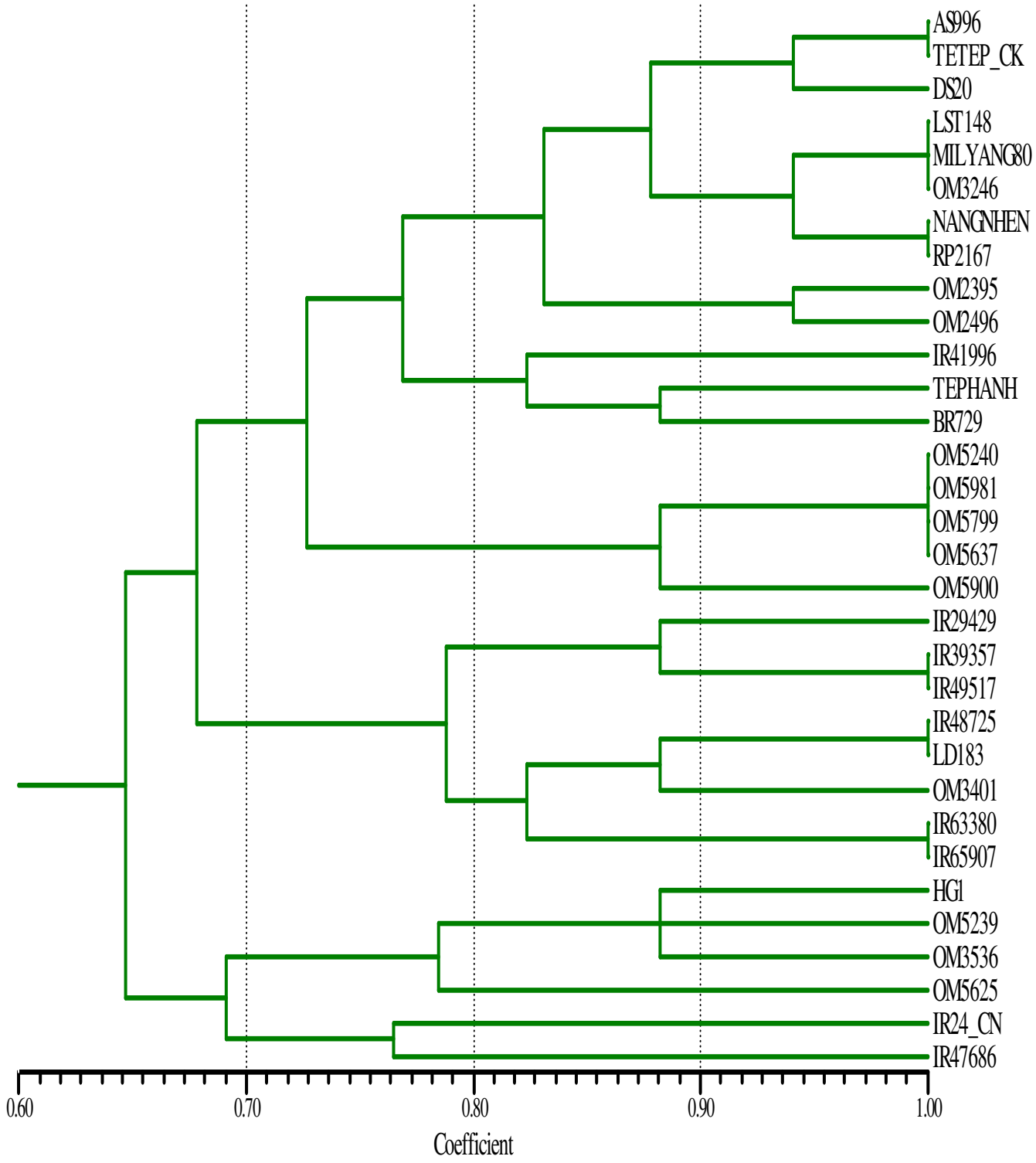

Figure 3b. Tree dendrogram shows genetic interaction of thirty – two varieties in term of genotype evaluation.

PCR based marker have opened up a new avenue to map genes of economic importance and the mapping of blast gene provided the opportunity for marker-assisted selection in hybrid rice. 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-

Table 4. Reaction of phenotype and genotype for the OM *P4, OM P 5, OM P 6, OMP 1 OM P 2* and *OMP 3* blast resistance locus.

No	Accession	Name	Race*OM P4*	Race*OM P5*	Race *OM P 6*	Race *OMP 1*	Race *0MP2*	Race *OMP 3*	RM 483	RG 64	RM21
1	1672	TaiNguyen	R	S	S	S	R	S	B	B	A
2	1694	NangThomChoDao	R	S	S	S	R	S	B	B	A
3	1559	NepDauKim	S	R	R	S	R	S	B	B	A
4	1564	Nep	S	R	R	S	R	S	B	B	A
5	1574	ThanNong Lun	S	R	R	S	R	S	B	B	A
6	1575	NangNgocTienNu	-S	R	R	S	R	S	B	B	A
7	1576	LunKienGiang1	S	R	R	S	R	S	B	B	A
8	1578	Nep Phu	S	R	R	S	R	S	B	B	A
9	1580	BaCo	S	R	R	S	R	S	B	B	A
10	1588	NepChuotChe	S	R	R	S	R	S	B	B	A
11	1591	Lua Mua504	S	R	R	S	R	S	B	B	A
12	1603	MotBuiDo	S	R	R	R	R	R	A	A	**B**
13	1606	ReHanh	S	R	R	S	R	S	B	B	A
14	1609	MotBuiMua	S	R	R	S	R	S	B	B	A
15	1610	VangNghe	S	R	R	S	R	S	B	B	A
16	1612	MotBuiTrang	S	R	R	S	R	S	B	B	A
17	1613	ThangCon	S	R	R	S	R	S	B	B	A
18	1614	NepAoVang	S	R	R	S	R	S	B	B	A
19	1618	Mashuri	S	R	R	S	R	S	B	B	A
20	1632	NepDaiLoan	S	R	R	S	R	S	B	B	A
21	1636	MotBui	S	R	R	S	R	S	B	B	A
22	1638	NangThomPhuocLy	S	R	R	S	R	S	B	B	A
23	1641	NangHuong2	-S	R	R	S	R	S	B	B	A
24	1643	NangThomGiua	-S	R	R	S	R	S	B	B	A
25	1652	NangThomChoDao	S	R	R	S	R	S	B	B	A
26	1658	NangThomGiau	-S	R	R	S	R	S	B	B	A
27	1664	NangTraRan	-S	R	R	S	R	S	B	B	A
28	1673	NangThom	-S	R	R	S	R	S	B	B	A
29	1676	SoiDa	-S	R	R	-S	R	S	B	B	A
30	1693	TauHuong	-S	R	R	-M	R	S	B	B	A
31	1696	TrangTep	S	R	R	R	R	S	A	B	A
32	1697	NeMuU	S	R	R	S	R	S	B	B	A
33	1700	NepRuoi Xanh	S	R	R	S	R	S	B	B	A
34	1702	NangLoanDoc	S	R	R	S	R	S	B	B	A
35	1703	TrangHoaBinh	S	R	R	S	R	S	B	B	A

Table 4. Contd.

36	1706	NepRuoi	S	R	R	S	R	S	B	B	A
37	1708	NangDen	S	R	R	S	S	S	B	B	A
38	1711	ChumDoc	S	R	R	S	S	S	B	B	A
39	1713	RuoiXanhDoc	S	R	R	S	S	S	B	B	A
40	1714	MuaDoc	S	R	R	S	S	S	B	B	A
41	1715	SoiDaDoc	S	R	R	S	-M	-M	B	B	A
42	1716	Nmang Thom Doc	S	R	R	S	-M	-M	B	B	A
43	1722	NangLoanDoc	S	R	R	S	S	S	B	B	A
44	1724	LemBuiDoc	S	R	R	S	S	S	B	B	A
45	1725	TrangTepDoc	S	R	R	S	S	S	B	B	A
46	1727	NepMuUDoc	M	R	R	S	M	M	B	B	A
47	1729	NepMoDoc	S	R	R	S	S	S	B	B	A
48	1730	NangHuongDoc	S	R	R	S	S	S	B	B	A
49	1666	NangHuong	R	R	R	S	S	S	B	B	A
50	1687	LemBui	R	R	R	S	S	S	B	B	A
51	1689	SoiDa	R	R	R	R	R	R	A	A	B
52	1704	NangTra	R	R	R	S	S	S	B	B	A
53	1710	NangTraRanDoc	R	R	R	S	S	S	B	B	A
54	1800	TeTep	R	R	R	R	R	R	A	A	A
55	1536	Trang Tep	S	S	S	S	S	S	B	B	A
56	1544	Nang Huong	S	S	S	S	S	S	B	B	A
57	1545	B40	S	S	S	S	S	S	B	B	A
58	1551	Nep Tuong	S	S	S	S	S	S	B	B	A
59	1557	Lun Thong	S	S	S	S	S	S	B	B	A
60	1560	MotBuiĐo	S	S	S	S	S	S	B	B	A
61	1567	TrangTron	S	S	S	S	S	S	B	B	A
62	1579	LunKienGiang2	S	S	S	S	S	S	B	B	A
63	1585	TroiCho	S	S	S	S	S	S	B	B	A
64	1586	NepBaTap	S	S	S	S	S	S	B	B	A
65	1587	KT15	S	S	S	S	S	S	B	B	A
66	1591	LuaMua504	R	R	R	R	R	R	A	A	A
67	1592	NgocNu	S	S	S	S	S	S	B	B	A
68	1593	TrangPhieu	S	S	S	S	S	S	B	B	A
69	1597	MongChimRoi	S	S	S	S	S	S	B	B	A
70	1600	NepMauLuon	S	S	S	S	S	S	B	B	A

Table 4. Contd.

71	1601	LunVangSom	S	S	S	S	S	S	B	B	A
72	1602	MongChimO	S	S	S	S	S	S	B	B	A
73	1606	ReHanh	R	R	R	R	R	R	A	B	A
74	1609	MotBuiMua	R	R	R	R	R	R	A	B	A
75	1612	MotButTrang	R	R	R	R	R	R	A	B	A
76	1614	NepAoVang	R	R	R	R	R	R	A	B	A
77	1620	NangNhenThom	S	S	S	S	S	S	B	B	A
78	1621	BaThiet	S	S	S	S	S	S	B	B	A
79	1636	MotBui	R	R	R	R	R	R	A	B	A
80	1640	DiTruyen2	S	S	S	S	S	S	B	B	A
81	1643	NangThomGiua	R	R	R	R	R	R	A	A	A
82	1645	NangThomSom	S	S	S	S	S	S	B	B	A
83	1646	NhoThom	S	S	S	S	S	S	B	B	A
84	1648	NangHuong	S	S	S	S	S	S	B	B	A
85	1650	MotBuiLun	S	S	S	S	S	S	B	B	A
86	1652	NangThomChoĐao	R	R	R	S	S	S	B	B	A
87	1656	TaiNguyen	S	S	S	S	S	S	B	B	A
88	1658	NangThomGiua	R	R	R	S	R	R	B	B	B
89	1660	TieuĐoi	S	S	S	S	S	S	B	B	A
90	1667	NangThom	S	S	S	S	S	S	B	B	A
91	1671	NangQuot	S	R	S	S	S	S	B	B	A
92	1675	NangTraRan	S	S	S	S	S	S	B	B	A
93	1677	NepMo	S	S	S	S	S	S	B	B	A
94	1683	NongNghiệpChum	S	S	S	S	S	S	B	B	A
95	1684	NepRuoiXanh	S	S	S	S	S	S	B	B	A
96	1686	NepNgheAn	S	S	S	S	S	S	B	A	A
97	1689	SoiĐa	R	R	R	R	R	R	A	B	A
98	1690	NangThom	S	S	S	S	S	S	B	B	A
99	1698	TauHuongVoTrang	S	S	S	S	S	S	B	B	A
100	1715	SoiĐaĐoc	R	R	R	S	R	R	B	A	A
101	1729	NepMoĐoc	R	R	R	S	R	R	B	A	A
102	1731	TrangHoaBinhĐoc	S	S	S	S	S	S	B	B	A
102	1731	TrangHoaBinhĐoc	S	S	S	S	S	S	B	B	A
103	1736	NanhChon	S	S	S	S	S	S	B	B	A
104	1737	NepVoĐen	S	S	S	S	S	S	B	B	A
105		IR 24	S	S	S	S	S	S	B	B	A

	Race OM P4	Race OM P5	Race OM P 6	Race OMP 1	Race 0MP2	Race OMP 3
S	20	65	64	12	49	14
R	85	40	41	93	56	91

S: Supceptible. R: Resistance, M: Medium.

Table 5. Blast resistance genes estimated and identified in CLRRI variety group.

Variety group	Cultivars	Reaction pattern
A	IR 24 IR 47686	OM P2
B	HG 1 OM 5239 OM 3536 OM 5625 IR 29426	OM P1
C	IR 39357 IR 49517 IR 48725 LD 183 OM 3401 IR 63380 IR 65907	OM P3
D	AS 996 Te Tep	OMP 1, OMP 2, OMP 3

adapted cultivars is also anticipated. The use PCR technology to rapidly amplify genomic DNA fragment will a significant.

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