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GENOTYPE ANALYSIS TO BLAST DISEASE IN RICE (*Oryza sativa* L.) IN MEKONG DELTA

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ABSTRACT

Blast, caused by Pyricularia grisea Cav., is one of the major fungal diseases infected rice (Oryza sativa L.) in Vietnam. This disease occurs to cause 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. The breeding program was aimed at improving blast resistance in high yielding varieties.

The six crosses OM24/IR64, IR24/OM2514, C53/IR 64, C53/OM 2514, OM1308/TeTep and IR36/C53 were conducted. Four cross were mapped using molecular markers. The resistance genes are inherited by dominant allele and located on chromosome 6, chromosome 8 and chromosome 11. Simple sequence repeat (SSR) marker as RM483 was used to detect 100 local varieties to find resistance to some race in Mekong delta. Phenotypic selection was used to compare the genotypes to check how accurate the polymorphisms in varieties. It showed that marker assisted selection (MAS) reached an accuracy of 100% in SSR marker RM483. These methods are accurate enough to apply in practice to select varieties that have blast resistance genes for breeding rice. Nang Huong, Trang Phieu, Hai Hoanh, Than Nong Lun, Nep Vo Dua, Trang Tep, Nep Do, Mong Chim Vang Nghe and Nep Chuot che exhibited their resistance to race P (OM 1). 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. These methods are accurate enough to apply in practice to select varieties that have blast resistance genes for breeding rice. Nang Huong, Lem Bui, Soi Da, Nang Tra, Nang Tra Ran Doc, Soc Nau, ***exhibited*** *their blast resistance to three isolates as OMP4, OMP5, OMP6. Three varieties : Te Tep, Soi da and Mot Bui Do resist to 6 isolates as OMP1, OMP2, OMP3, OMP4, OMP5, and OMP6. These are considered as valuable materials for pyramiding resistance genes to create durable resistant varieties.*

Keys words: Blast resistance, *Oryza sativa*, PCR, SSR, STS markers

NTRODUCTION

Rice blast disease, cause by *Pyricularia grisea* Cav., is one of the most devastating diseases of rice (Ou 1985),. This is one of major rice diseases in Vietnam - the most destructive diseases of rice throughout the world. In some areas of Vietnam, the potential to reduce yield by more than 20%. The first gene for resistance to blast, *Pi-a,* was identified by Shinoda et al. (1971). Since then, more than 50 genes for resitance to blast have been identified. The work of Kiyosawa (1981) established the basis for genetic studies of blast resistance genes in japonica rice using Japanese blast strains. Development of near isogenic lines improved the situation (Bonmam and Mackill 1988). So far, bast disease has been evaluated among 922 rice varieties, which collected from many countries and these were classified into six variety groups (Yoshimich Fukuta et al. 2007). Races or clusters of races of blast have been identified through classical genetic analysis. The existence of many different races of *Pyricularia grisea* has been making it difficult to develop durable and long-lasting resistant varieties. The relationships between resistance genes in rice and virulence gene in blast pathogen have not been

clarified yet, due to lack of universal differential varieties, which could identify the true reactions of pathogen without the influences of genetic backgrounds of rice. Moreover, the research on diversity of blast races and resistance genes has been carried out on relatively small area or country basis, without global view. The genetic constitution for blast resistannce 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. As many as genes conferring resistance blast have been identified to various races of the pathogen. 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. Although some oustanding work has been done in the field, rice disease investigation in Vietnam characterized chemical control. However, resistant varieties often break down within three to five years after releasing for cultivation. To breed rice varieties with more durable blast resistance, multiple resistance genes must be incorporate 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 marker assisted selection and pyramiding of useful genes. Some markers for blast resistance – *Pi-1, Pi 2(t),* ***Pita, Pit****, Pi7(t), Pi9(t), Pi1(t) and* ***Pib*** are available for molecular maps (Khush and Brar 2004), In the present article, the development of molecular markers for selection of resistance to mono race is a goal of rice breeding program.

MATERIAL AND METHODS

Collection of diseased rice samples

Blast infected leaves with typical lesions were collected from farmer fields at irrigated regions in six provinces of Mekong Delta (MD) area (namely at CanTho = race OMP1), AnGiang=OMP2, Long An=OMP3, Dong Thap=OMP4, Hau Giang= OMP5 and Vinh Long =OMP6) from 2005 Dong Xuan season. All monocultures were maintained in suitable medium for long term storage at CLRRI Genebank.

Genetic materials and disease evaluation

Six crosses involving three selected different resistance varieties (table 1) among 100 local varieties, and 32 improved varieties were implemented. Te Tep and IR24 were used as checks.

The138 F_2 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. Twenty one day old seedling were inoculated with blast isolates *OMP1, OMP2, OMP3, OMP4, OMP5, and OMP6*. The rice seedling were sprayed with 50ml of blast inoculum suspension per tray (5 x $\mathbf{10^4}$ condia/ml) and incubated for 24 hours in a controlled temperature of 25^{o}C. The seedlings were then transferred into a temperature and humidity controlled chamber. Plants were observed for blast disease symptoms for 1 week after inoculation. They were scored for blast reaction when the typical lesions developed on the susceptible parental cultivar IR24. This phenotypic evaluation was repeated three times.

Rapid isolation of rice DNA:

Isolate DNA for PCR analysis, which does not require liquid nitrogen, needs only small amount of tissue sample and protocols, was done by Lang (2002). DNA suitable for PCR analysis was prepared using a miniscale procedure in a labeled 1.5 ml centrifuge tube 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). Grinding 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. 400µ l 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 1µ l is sufficient for PCR analysis. DNA quality and quantity were spectrophotometrically determined.

PCR amplification:

The genomic DNA from both of the 148 F_2 plants and the 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 25µ l]. Template DNA were initially denatured at 94^{o}C for 5 min, followed by 30 cycles of PCR amplification under the following parameters, 1 min denaturation at 94^{o}C, 1 min primer annealing at 55^{o}C and 2 min. primer extension at 72^{o}C. Final 5 min incubation at 72^{o}C was allowed for completion of primer extension on a 480-thermalcycler. The amplified products were electrophoretically resolved on 1.2 % agarose and 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 were used for determining linkage groups and for the calculation of map distance in JOINMAPV.14. The segregation ratios of individual markers were calculate with the software program QGENE V.2.0 (Nelson 1994), and skewing was indicated when the ratio deviated significantly from the expected.

RESULTS AND DISCUSION

Resistance tests and genetic mapping

Analysis on the F_2 population

DNA samples from the 457 F_2 populations included 109 plants from IR24/IR64, 42 from IR24/ OM2514 and others were assessed through PCR amplifcation using 13 primers (RM225, RM314, RM111, RM253, RM50, RM276, RM136, RM83, RM541, RM162, RM276, RM343 and RM30) on chromsome 6. The 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 individuals could be classified into homozygote for the IR24 type marker size 210bp fragment, homozygote for IR64 type marker 200 bp fragment at the locus RM162.

In the first step, resistance reaction of 457 F_2 plants 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 gene for all the resistant parents. Infection rates varied 92-98% among the progeny. Resistant F_2 plants were continued to be tested in F_3 generation. Segregation ratios for resistance to blast were summarized in table 1.

According to the resistance data from the F_2 generation of IR24/IR64 and IR24/OM2514, phenotypic pools were set. In the fist step, mapped molecular markers were set up with 16 SSR markers in chromosome 6 until a region, which was identified in each cross where the markers showed a clear differentiation between resistance and susceptible (figure 1)

R Gene to race *OMP1*

The 16 codominant SSR markers have been mapped in both two populations at a distance of 3.9 cM and 3.0 cM in IR24/IR64 (102 F_2) and IR24/OM2514 (46 F_2), respectively (figure 1).

R Gene to race *OMP2*

The resistance gene has been localized on chromosome 11 with 9 markers (RM167, RM552, RM120, RM441, RM479, RM202, RM21, RM206, RM224). It linked to marker RM21 in population F_2 of C 53/OM2514 at distance of 7.7cM (figure 2)

R Gene to race *OMP3*

In the F_2 from IR36/C53, resistance to race OMP3 was detected by polymorphism of 11 SSR markers (RM310, RM344, RM483, RM88, RM42, RM223, RM515, RM284, RM256, RM215 and RM230) on chromosome 8. Among them, RM483 linked to the R gene at a distance of 0.7cM (figure 2).

Table 3.1. Progenies studied for genetic analysis of resistance to blast

Cross	Resistant parent	Isolate	Susceptible parent	No. of progeny plants	Segregation R:S	Chi-square test (3:1)	Infection rate (%)
IR24/IR64	IR64	OMP1	IR24	109 F_2	79:30	0.25	95
IR24/OM2514	OM 2514	OMP1	IR24	46F_2	35:11	0	98
C53/ IR64	IR64	OMP2	-	61F_2	46:15	0.003	92
C53/ OM2514	OM 2514	OMP2	-	101F_2	84:17	2.56	92
IR36/ C53	C53	OMP1, and OMP2	IR36	48F_2	36:12	0	95
OM1308/Te Tep Total	TeTep	OMP1, OMP2,	OM1308	92 F_2, 56BC_1F_2 457 F_1 and 56BC_1F_2	83:16	2.56	92

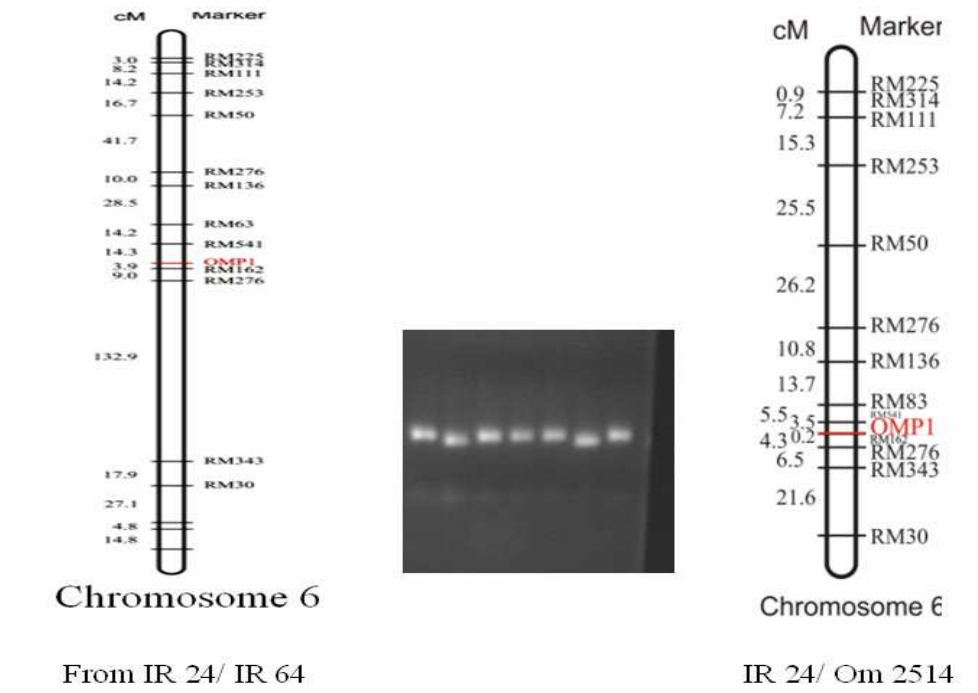

Figure 1. Molecular linkage maps of chromosomal regions harbouring the resistance gene *OMp-1*

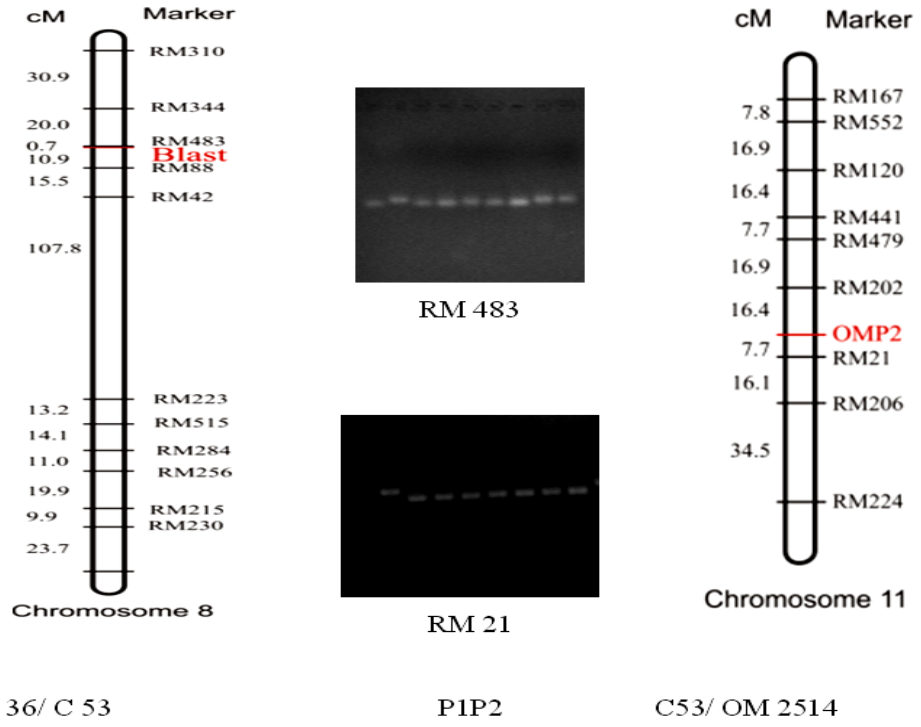

Figure 2 : Molecular linkage maps of chromosomal regions harbouring the resistance gene *OMp-2 and OMp1 + OMp 2*

Molecular markers for breeding strategies

DNA markers have been identified. They are useful for marker-assisted selection in breeding programs for the introgression of resistance genes from donors into high yielding cultivars. SSR markers are linked R genes to blast races *OMP1, OMP2, OMP3* in Vietnam in the coupling phase and therefore are suitable for selecting homozygous resistance as well as heterozygous susceptible plants, avoiding the necessity of two backcrossing steps, which would be indispensable to identify carriers of dominance genes in classical breeding programs. Similarly, SSR marker RM21 is useful for the identification of lines carrying blast resistance genes. This is reference with C53/OM2514 in the homozygous or heterozygous state. On the other hand, RM162 is linked to OMP1 in repulsion and hence allows selection of homozygous resistant lines, which are characterized by the lack of the SSR fragments. In this case, however, samples that failed to amplify cannot be differentiated from resistant plants. Therefore, stringent controls have to be included into the experimental design.

Some primers designed form RG64 on chromosome 6 linked to R gene in F_2 population of OM 1308/Te Tep (Lang et al 2001). The population was evaluated and identified resistance plants to race OMP4 based on RG 64 marker. This indicated a matching of up to 96.49%.The accuracy of identifying individuals carrying the resistance gene by selecting from 92 lines F_2: 37/92 R and 43/92 S and 12/92 MR (heterozygotes). In this population, a series of microsatellites markers in close linkage to resistance genes is effective against blast. SSR primers (RM162, RM541) can detect R plants in F_2 (92 plants) and BC_1F_2 (56 plants) from OM1308/TeTep. There may be different 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. They were resistant in the field under natural infection conditions but become susceptible in the greenhouse upon mechanical inoculation of the leaves.

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 to identify blast genes in a segregating population of 71 F_3 individuals (OM1308/Te Tep) at the locus RM162 (table 3.3)

Table 3.3. Prediction of genotypes based on RM162 locus to OMP1 and OMP4 in F_3 population of OM1308/Te Tep

Phenotype	No.Plants	Genotype		Accuracy (%)
		R	S	
OMP4				
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

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 genebanks. Blast resistance genotypes was measured for four microsatellites (RM 21, RM162, RM483 and one STS marker (RG64F-R). One of the blast resistance genes mapped in rice *Pi-2(t)* was mapped on chromosome 6 (Mackill and Bonman 1992), and located 2.8 cM distance from the RFLP marker RG64 (Yu et al. 1991). Several DNA markers have been identified. They are useful for marker-assisted selection in breeding program for the introgression of resistance genes from donor lines into adapted germplasm. 100 local varieties in CLRRI GeneBank were used. It included 57 R accessions and 43 S to 6 races OMP1, OMP2, OMP3, OMP4, OMP5, and OMP6).

The accuracy of identifying individuals carrying the resistance gene by selecting from 105 local varieties. Comparison genotype from phenotype expressed 57% R and 43% S (Table 3.4). The results that **all 105** lines detected by sequence tagged site marker (RG 64F-R) with the same phenotypic selection were used to compare with genotype to check accurate 60 % with RG 64 and race OMP4.The methods the results were polymorphisms in varieties show that marker-assisted selection (MAS) reached an accuracy of 100% in SSR marker with RM483. These methods are enough accurate to apply in practice to select varieties that have blast resistance genes for breeding rice. Nang Huong, Trang Phieu, Hai Hoanh, Than Nong Lun, Nep Vo Dua, Trang Tep, Nep Do, Mong Chim Vang Nghe and Nep Chuot che exhibited R to race OMP1. Phenotypic selection was used to compare with appropriate genotype to check the accuracy. Polymorphisms among varieties show that MAS reached an accuracy of 99.49% at the locus RM21. These methods are enough accurary to apply in practice to select varieties that contain blast resistance genes for breeding application. Nang Huong, Lem Bui, Soi Da, Nang Tra, Nang Tra Ran Doc, Soc Nau, and Te Tep resist to three races as OMP4, OMP5, OMP6. Then Te tep, Soi da resist to OMP1, OMP2, OMP3, OMP4, OMP5, and OMP6. These are considered as valuable materials for pyramiding resistance genes to created durably resistant varieties (table 3.4).

Table 3.4. Reaction of phenotype and genotype for the OMP4, OMP5, OMP6, OMP1 OMP2 and OMP3 blast resistance locus.

No	Acce-ssion	Name	Race OMP4	Race OMP5	Race OMP6	Race OMP1	Race 0MP2	Race OMP3	RM483	RG64	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	Mahsuri	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

No	Acce-ssion	Name	Race OMP4	Race OMP5	Race OMP6	Race OMP1	Race 0MP2	Race OMP3	RM483	RG64	RM21
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	Nang 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
71	1601	LunVangSom	S	S	S	S	S	S	B	B	A
72	1602	MongChimO	S	S	S	S	S	S	B	B	A

No	Acce-ssion	Name	Race OMP4	Race OMP5	Race OMP6	Race OMP1	Race 0MP2	Race OMP3	RM483	RG64	RM21
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	MotBuiTrang	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
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 OMP4	Race OMP5	Race OMP6	Race OMP1	Race OMP2	Race OMP3
S	**20**	**65**	**64**	**12**	**49**	**14**
R	**85**	**40**	**41**	**93**	**56**	**91**

S: Supceptible. R: Resistance : M: Medium

The diversity of 32 varieties in blast resistant genotypes was also measured by 34 polymorphic SSR markers. However some primers (e.g. three microsatellite and one STS as RM21, RM162, RM483, RG64F-R) exhibited strong polymorphism. A similarity matrix based on pair wise comparisons of pooled data was made using NTSYSpc. The dendrogram showed four major clusters among 32 varieties (Figure 3) and table 5. AS996 and Te Tep belonged to cluster D together distinct from all others accessions at 60% similarity index. Three allelic conditions of the plants susceptible to the disease were detected: homozygotes for resistance allele, homozygotes for susceptible allele and heterozygotes. The detection of individuals of homozygotes for susceptible allele and heterozygotes was shown.

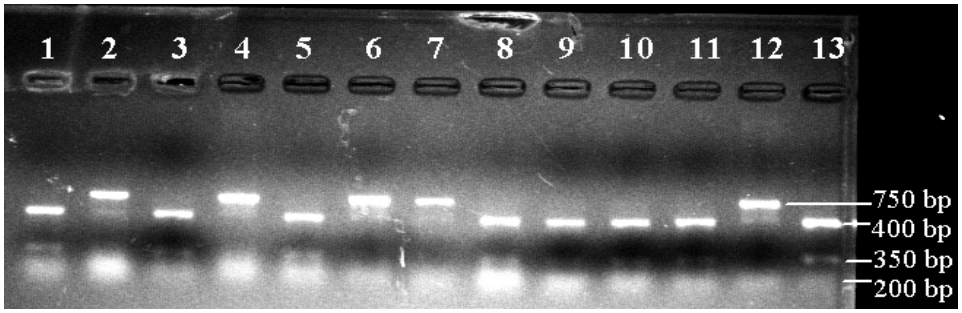

Figure 3.3 PCR production of the local varieties based on the banding pattern of t**he** RG64 locus digested with *Hea*III, gel agarose 1.5 %, TAE 1X. M: marker: kb ladder: lines: 1: Te Tep, 2: IR64, 3: Soc Nau, 4: Tiêu Đôi, 5: Tai Nguyen, 6: Nang Quot, 7: Nep Mu U, 8: Lem Bụi, 9: Soi Da, 10: Nang Tra, 11: Nang Tra Ran Doc, 12: Nang Huong, 13: Nang Thom Cho Dao.

Table 3.5. Blast resistance genes estimated and identified to each isolate

Genetic Cluster	Cultivars	Reaction pattern to
A	IR24	OMP2
	IR47686	
B	HG1	OMP1
	OM5239	
	OM3536	
	OM5625	
C	IR29426	OMP3
	IR39357	
	IR49517	
	IR48725	
	LD183	
	OM3401	
	IR63380	
	IR65907	
D	AS996 Te Tep	OMP1, OMP2, OMP3

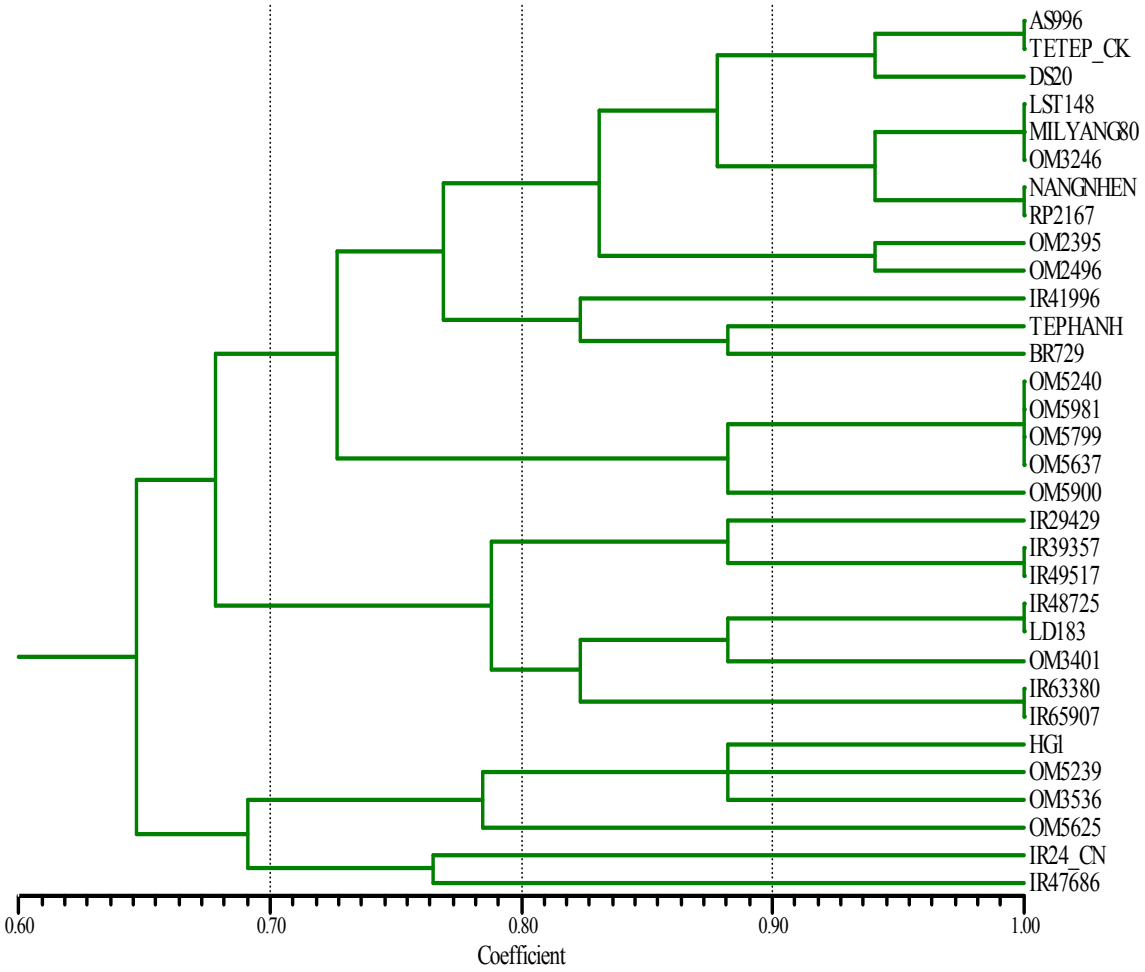

Figure 3.4. Tree dendrogram shows genetic interaction of thirty – two varieties in term genotype evaluation

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 resistance gene to blast in the field, which has been previously mapped at RM162 locus. 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. PCR based markers have opened up a new avenue to map genes of economic importance. The mapping of gene blast provided the oppoturnity for marker-assisted selection in high yielding rices.

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 anticifcited. The use PCR technology to rapidly amplify genomic DNA fragment will a significant.

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Phân tích tính kháng bệnh đạo ôn đối với từng nòi riêng biệt ở đồng bằng sông Cửu Long

Bệnh đạo ôn do nấm *Pyricularia grisea* Cav., gây ra; là một trong những đối tượng nguy hại nhất trong sản xuất lúa gạo của Việt Nam. Năng suất có thể thất thu trên 20%. Giống bản địa được khai thác như nguồn cung cấp gen kháng trong chương trình cải tiến giống lúa cao sản kháng bệnh đạo ôn

Thực hiện 6 cặp lai để phân tích di truyền là OM 24/IR 64, IR 24/OM 2514, C53/IR 64, C53/OM 2514, OM1308/TeTep và IR36/C 53. Bốn cặp lai được khai thác để lập bản đồ di truyền với chỉ thị SSR, đặc biệt nhóm gen trội định vị trên nhiễm sắc thể số 6. 8 và 11. RM483 được khai thác và phát hiện 100 giống lúa bản địa biểu thị kháng rộng với các nòi nấm được thu thập ở ĐBSCL. Đó là Nàng Hương, Trắng Phiếu, Hai Hoành, Thần Nông Lùn, Nếp Võ Dừa, Trắng Tép, Nếp Đỏ, Móng Chim Vàng Nghệ and Nếp Chuột Chê kháng với nòi OMP1. Chỉ thị STS được thiết kế mồi từ RG64 đạt chính xác 99,49% khi so sánh giữa kiểu gen và kiểu hình tương tự như RM21 trên nhiễm sắc thể số 6. Nàng Hương, Lem Bụi, Sỏi Đá, Nàng Trá, Rằn Đốc, Sóc nâu thể hiện tính kháng với các nòi OMP4, OMP5, OMP6. Ba giống bản địa Tẻ Tép, Sỏi Đá và Một Bụi Đỏ kháng được cả 6 nòi OMP1, OMP2, OMP3, OMP4, OMP5, và OMP6. Đây là nguồn cung cấp gen kháng với phổ kháng rộng rất cần thiết cho chương trình lai tạo giống lúa.