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Full Length Research Paper

Identification of blast resistance expression in rice genotypes using molecular markers (RAPD & SCAR)

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Rice is the second most important cereal crop of developing countries and the staple food of about 65% of the world's population. In this endeavor, it is important to identify resistant gene(s) with the help of markers. Once a gene is tagged with a molecular marker, it can be transferred selectively into different genetic backgrounds by marker assisted selection. For this purpose, 48 elite Indian and exotic rice genotypes were evaluated for resistance to blast disease under induced epiphytotic conditions obtained in the field. The disease severity (%) and AUDPC was less than 45% and 1000, respectively, in all the resistant genotypes, while it was around 85% and higher than 2000 in the case of susceptible genotypes, respectively. Substantial variability was present among rice genotypes for resistance to *Magnaporthe grisea.* Ten random amplified polymorphic DNAs (RAPD) and two sequence characterized amplified region (SCAR) primers were used to identify blast resistant genes. Markers OPA-05, OPF-06, OPF-09, OPF-17, OPG-17, OPG-18, OPG-19, OPH-18, OPK-12, P-265-550 and P-286-350 found linked to blast resistance in most of the resistant genotypes could be considered as potent molecular markers in the selection of blast resistant genotypes. Amplification with RAPD and SCAR primers revealed a non-allelic relationship among resistant genotypes and thus, there is a good possibility of obtaining enhanced resistance through gene pyramiding.

Key words: Rice, molecular markers, *Magnaporthe grisea*, RAPD, SCAR, disease severity, AUDPC.

INTRODUCTION

Rice (*Oryza sativa*) is the second most important cereal crop of developing countries and the staple food of about 65% of the world's population. The production of rice to be achieved by 2020 is 128 million tones to feed the growing population in India. Rice blast caused by *Magnaporthe grisea* (anamorph: *Pyricularia oryzae*) is one of the most damaging and therefore, an important diseases of rice in many parts of the world. The disease is common where rice is grown between 9° and 45° N latitude. The disease occurs on leaves, stems and seeds of the cultivated crop. Estimated yield losses in rice due to blast disease are reported to vary between 20 - 60% (Xu et al., 1995). Maximum losses measured in susceptible checks were 20.9 % in IR50 in the Philippines and 50.2% in Daechang in Korea (Lee et al., 1990). Padmanabhan (1965) reported that *P. oryzae* has a specific and large host range; almost all the plants of the family Poaceacae. Breeding progress for blast resistance in rice is not very satisfactory due to lack of knowledge about the fast development of most aggressive isolates of *M. grisea* (syn *P. oryzae*) and the non availability of suit-able molecular markers linked to blast resistance genes. Although 30 resistance genes, quantitative as well as qualitative to blast pathogen have been identified so far in different parts of world (Ahn and Ou, 1982; Ou, 1980; Villarreal et al., 1981), the objective to develop durable blast resistance cultivars can be accomplished by pyramiding blast resistance genes. Phenotypic selection cannot be used for pyramid resistance genes because the presence of one major gene obscures the effect of other genes and

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they are time consuming, labour intensive and frequently inconclusive because of environmental effects on the expression of susceptibility.

Molecular markers linked to major blast resistance genes offer a powerful tool for marker aided indirect selection of resistance loci in gene pyramiding strategies. random amplified polymorphic DNAs (RAPD) and sequence characterized amplified region (SCAR) technologies provide the researcher with a quick and efficient screening methodology for the detection of DNA sequences based polymorphism at variable numbers of loci. RAPD has been used to construct genetic maps and for the molecular tagging of various agronomic traits in different crop species (William et al., 1993; Sandhu et al., 2003). A number of blast resistance genes have been mapped relative to tightly linked RAPD and RFLP markers in breeding, however, indirect selection through marker assisted selection (MAS) is not well documented (Naqvi and Chhatoo, 1996). Once a gene is tagged with a molecular marker, it can be transferred selectively into different genetic background by marker-assisted selection (Liu et al., 1999). The increasing threat of rice blast disease calls for serious efforts to develop resistant rice varieties against this pathogen. In this endeavor, rice cultivars were screened with the help of known RAPD and SCAR markers for identifying blast resistant gene(s).

MATERIALS AND METHODS

Plant material and field trials

48 elite Indian and exotic rice genotypes were evaluated for resistance to blast disease under induced epiphytotic conditions obtained in the field at Meerut (North West Plains Zone, India, 28.99°N and 77.70°E) in the years 2005 - 2006 and 2006 - 2007. Approximately, 25 - 30 plants of each genotype were grown in each row of a plot consisting of 3 rows (3 m each; where a 20 cm plant to plant and 25 cm row to row distance was maintained). Genotype Pusa Basmati-1, a highly susceptible rice genotype to blast pathogen was planted in alleys and borders to enhance the spread of inoculums. Standard agronomic management practices were followed to raise a healthy crop. Inoculums were prepared from infected leaf samples having conidia and mycelium of blast pathogen. Inoculums having concentrations of 10×10^4 to 50×10^4 conidia/ml were used for the inoculation of plants. Fields were frequently irrigated to induce environmental conditions conducive to blast pathogen. Leaves were harvested from 15 days old seedling of the 48 genotypes from field trials during 2006 - 2007 Kharif season. Then, the leaf samples were packed into poly bags and stored at -80°C for the purpose of isolation of genomic DNA, using CTAB method (Moller et al., 1992).

Field data analysis and evaluation for the presence of molecular markers

Disease severity (%) was measured following the methodology of Jeger (2004). Disease severity (%) was recorded at 3 different stages viz: late anthesis, late milking and dough stages. Area under disease progress curve (AUDPC) based on disease severity over time, which has been suggested to be a pragmatic approach for disease assessment was estimated using the formula:

$$\text{AUDPC} = \sum_{i=1}^{i} [\{(Y_i + Y_{(i+1)})/2\} \times (t_{(i+1)} - t_i)]$$

where Y = disease level at time t_i, and $t_{(i+1)} - t_i$ = duration (days) between two disease score Analysis of variance (ANOVA) for disease severity (%) at the dough stage and AUDPC was performed using SAS software (version 603; SAS Institute Inc; CaryNC 1997). For the purpose of validation of molecular markers in both cases that is, RAPD as well as SCAR, all the genotypes were evaluated for the presence of marker bands supposed to be linked with blast resistant gene(s).

DNA amplification and gel electrophoresis

Genotypes were subjected to screen for the resistance genes with the help of 10 RAPD and 2 SCAR primers. Each reaction mixture (30 µl) used for RAPD and SCAR amplification consisted of an assay buffer (10 mM Tris HCl, pH 8.0; 50 mM KCl), 3.0 mM $MgCl_2$, 1 U of Taq DNA polymerase, 1.0 mM each of dATP dTTP, dCTP and dGTP, 10 µl/m of primer (Bangalore Genei) and approximately 50 and 250 ng of genomic DNA for RAPD and SCAR, respectively. The PCR amplification conditions for RAPD analysis were as follows: initial extended step of denaturation at 95°C for 4 min followed by 45 cycles of denaturation at 95°C for 1 min, primer annealing at 30°C for 1 min, elongation at 72°C for 2 min, followed by extension step at 72°C for 7 min and then final hold at 4°C till electrophoresis. For SCAR analysis, the number of cycles was 35 and annealing temperature was 50°C. PCR products were mixed with 5µl of gel loading dye (1x buffer, bromophenol blue, 0.1%; xylene cyanol 0.1%; and glycerol in water, 50%). The amplification products were electrophoresed on 1.5% agarose gel at 3 - 5 volts/cm in 1x TBE buffer. Genomic DNA was quantified by UV absorbance at 260 and 280 nm, using UV-VIS eppendorf spectrophotometer. The ratio of OD 260/280 was also calculated to estimate the purity of nucleic acid. Genomic DNA was also quantified by agarose gel electrophoresis. As the size of the genomic DNA is quite big, a 0.8% gel was used to visualize it, as it can resolve DNA molecules in the range of 0.7 - 8.5 kb.

RESULTS AND DISCUSSION

48 genotypes were investigated for disease resistance based on disease severity (%) at the dough stage and through AUDPC during 2005 -2006 and 2006 - 2007 Kharif seasons. The disease severity (%) at the dough stage in some of the genotypes was less than 45% in both years and thus considered as resistant, while those genotypes which showed around 85% were considered as susceptible. AUDPC based on disease severity data recorded at three growth stages; late anthesis, late milking and dough stages, was less than 1000 in all the resistant genotypes, while it was more than 2000 in the case of susceptible genotypes (Table 1).

Based on disease reaction (disease severity (%) and AUDPC), all rice genotypes were identified as resistant, moderately resistant, moderately susceptible and susceptible following the disease rating scale of Naqvi and Chattoo (1996) and Villareal et al. (1981). Artificial disease pressure was created by inoculating all rice genotypes with the most aggressive isolates identified at

Table 1. Percent blast disease scores for resistant and susceptible genotypes in the genetic analysis.

Genotype	Mean disease response to blast			
	% Severity		AUDPC	
	2005 - 2006	2006 - 2007	2005 - 2006	2006 - 2007
IR-72107-4-159-1-3-3-3	25.00 ± 2.58	20.00 ± 2.25	623.38 ± 87.35	648.75 ± 90.22
IR-74886-55-2-3-2	45.00 ± 2.77	50.00 ± 4.60	1134.38±100.38	1085.23 ± 99.62
IR-74371-70-11	35.00 ± 4.80	40.00 ± 3.75	909.38 ± 90.25	925.43 ± 98.97
PSBRC-80-1	30.00 ± 3.30	35.00 ± 3.20	825.00 ± 81.75	816.22 ± 85.22
IR-75298-59-3-1-3	25.00 ± 2.25	20.00 ± 2.30	796.88 ± 83.22	734.29 ± 88.86
IR-7388-1-2-7	50.00 ± 4.30	40.00 ± 4.75	1415.63 ± 107.65	1069.98 ± 104.46
IR-71604-4-1-4-7-10-2-1-3	45.00 ± 5.42	55.00 ± 4.95	1256.25 ± 101.22	1345.78 ± 109.76
IR-71527-44-1-1	25.00 ± 2.28	35.00 ± 3.46	684.38 ± 73.22	567.43 ± 70.21
BW-391	35.00 ± 3.50	45.00 ± 2.66	984.38 ± 78.88	923.54 ± 76.26
IR-80920	20.00 ± 2.60	15.00 ± 1.75	562.50 ± 47.22	456.09 ± 56.43
IR-80922	25.00 ± 2.22	35.00 ± 3.35	684.38 ± 42.66	568.53 ± 45.25
IR-71700-247-1-1-2	20.00 ± 2.15	30.00 ± 3.10	562.00.50 ± 47.33	531.34 ± 51.25
IR-74374-46-1-1	25.00 ± 2.21	30.00 ± 3.30	787.50 ± 85.89	690.70 ± 74.33
Vallabh-21	15.00 ± 3.70	25.00 ± 2.25	525.00 ± 40.89	548.56 ± 41.97
Basmati-370	70.00 ± 3.80	65.00 ± 3.51	1825.00 ± 100.75	1756.35 ± 100.02
Sugandha-4(APEDA)	75.00 ± 4.15	85.00 ± 4.65	1350.00 ± 96.54	1465.00 ± 98.87
SVT-16	50.00 ± 4.21	65.00 ± 3.52	1150.00 ± 121.03	1065.21 ± 118.95
Tarori	75.00 ± 4.20	85.00 ± 4.66	1925.00 ± 173.25	1876.57 ± 176.29
Tarori (APEDA)	70.00 ± 3.81	55.00 ± 5.57	1275.00 ± 93.45	1131.67 ± 120.04
SVT-28	65.00 ± 3.54	75.00 ± 4.22	1550.00 ± 125.87	1587.33 ± 132.89
Vallabh Basmati 21	70.00 ± 3.79	65.00 ± 3.51	1637.50 ± 165.85	1567.54 ± 155.19
P-79	45.00 ± 5.39	40.00 ± 2.55	1087.50± 98.89	982.56 ± 95.65
P-89	35.00 ± 3.50	25.00 ± 2.58	850.00 ± 82.80	756.20 ± 80.02
Pusa-1121	50.00 ± 4.19	65.00 ± 3.58	1200.00 ± 92.00	1319.54 ± 94.45
Super Basmati(APEDA)	70.00 ± 3.85	65.00 ± 3.61	1225.00 ± 109.89	1365.78 ± 102.87
HKR-1	80.00 ±4.41	75.00± 4.00	2300.00 ± 195.45	2278.15 ± 193.85
NDR-359	80.00 ± 4.45	85.00 ± 4.60	2062.50 ± 192.25	2126.71 ± 192.15
CSR-27	80.00 ± 4.46	85.00 ± 4.62	2062.50 ± 192.25	2098.81 ± 193.18
SVT-10	80.00 ± 4.47	75.00 ± 4.10	2012.50 ± 190.13	2143.67 ± 191.89
16-Macro	75.00 ± 4.13	85.00 ± 4.64	2137.50 ± 195.12	2089.67 ± 192.87
32-Macro	85.00 ± 4.63	75.00 ± 4.14	2437.50 ± 198.34	2540.80 ± 200.12
Tilakchandan	85.00 ± 4.68	90.00 ± 4.71	2462.50 ± 199.33	2545.10 ± 201.18
Sugandha-2	85.00 ± 4.64	75.00 ± 4.17	2175.00 ± 195.18	2190.79± 196.14
Saket-4	85.00 ± 4.64	80.00 ± 4.47	2012.50 ± 193.23	2200.00 ± 195.17
39-Macro	85.00 ± 4.62	75.00 ± 4.15	2337.50 ± 200.10	2465.70 ± 201.21
23-Macro	75.00 ± 4.17	80.00 ± 4.46	2100.00 ± 198.18	2186.65 ± 191.19
SVT-5	80.00 ± 4.45	70.00 ± 3.82	2337.50 ± 200.10	2378.67 ± 200.98
SVT-21	80.00 ± 4.46	75.00 ± 4.13	2312.50 ± 199.25	2235.76 ± 195.08
NDR-180	85.00 ± 4.65	75.00 ± 4.15	2462.50 ± 201.35	2564.35 ± 208.49
PB-1	85.00 ± 4.67	80.00 ± 4.50	2312.50 ± 199.25	2097.67 ± 195.80
MAUU-15B	75.00 ± 4.17	60.00 ± 3.46	2100.00 ± 199.19	2098.78 ± 198.35
NDR-18	80.00 ± 4.51	70.00 ± 3.82	2137.50 ± 200.80	2078.34 ± 198.37
Ajay*PB-1	80.00 ± 4.52	85.00 ± 461	2350.00 ± 211.10	2267.16 ± 209.08
P-83	80.00 ± 4.52	75.00 ± 4.18	2150.00 ± 201.80	2089.36 ± 198.88
P-111	80.00 ± 4.56	70.00 ± 3.81	2162.50± 201.89	2199.51 ± 202.10
P-31	75.00 ± 4.17	55.00 ± 4.26	2025.00 ± 195.51	2132.45 ± 198.54
Sathi	95.00 ± 6.51	85.00 ± 4.65	2762.50 ± 225.50	2645.31 ± 224.51
P-70	70.00 ± 3.79	80.00 ± 4.51	2050.00 ± 195.50	2156.44 ± 197.05

this centre. Moderately high correlation between the genotypes classified based on the percent disease score and AUDPC suggested that AUDPC was as much efficient as percent disease scoring used for genotype classifications. Presence of a continous range of variation for disease severity and AUDPC was an indication that blast resistance is under the control of several additive genes having small but cumulative effect on disease resistance. Singh and Rajaram (1991) reported similar additive gene action for the leaf rust of wheat. An earlier report (Sandhu et al., 2003; Naqvi and Chattoo, 1996) suggested a polygenic control for rice blast resistance. Due to the presence of minor genes, variation for disease reaction between two varieties was not significant. With increasing number of additive genes, variation among genotypes would increase. Additive gene action is always an attraction for breeders to exploit traits and bring desirable changes in the population through selection and accumulation of resistant genes into a single genotype.

However, the non-availability of reliable molecular markers linked to blast resistance due to selection for disease resistance at the morphological level is not very promising. Due to changes in environmental factors such as temperature, humidity and growth stages, disease expression can be altered and selection for disease resistance genotypes can be biased (Pengyuan et al., 2004). In this scenario molecular markers, which are not influenced by environmental changes may play a very vital role for the successful identification of the presence of blast resistant genotypes.

Primer screening

10 RAPD primers and 2 SCAR primers were used to detect polymorphism among 48 rice genotypes. Out of these, 8 RAPD and 2 SCAR markers produced distinct, reproducible and polymorphic profiles and displayed linkages in the coupling phase to blast resistance genes. These results were reported by Sandhu et al. (2003) and Naqvi and Chhattoo (1996).

The approximate size range of the RAPD products was 40 bp to 4.2 kb. Reproducibility of the amplification pattern was checked by repeating each reaction at least twice without deliberate alteration in the protocol. Although, a number of species-diagnostic RAPD bands were noted, most of them were either rather faint or not repeatedly found in all the resistant genotypes. Thus, a large number of potentially genotype-specific, informative RAPD bands were eliminated from consideration. The rate of polymorphism was highest in the case of RAPD primer OPF-06 followed by OPA-05 and OPH-18. However, there was no amplification observed in case of OPF-19. Comparing between banding pattern of resistant and susceptible genotypes, 11 fragments produced by OPA-05 (1000 and 1200 bp) (Figure 1), OPF-06 (4000 bp), OPF-09 (600 bp), OPF-17 (700 bp), OPF-19 (no band), OPG-17 (100 bp), OPG-18 (550 bp), OPG-19 (500 bp), OPH-18 (100 and 500 bp) (Figure 2) and OPK-12 (900 bp) were identified and linked with blast resistance and thus, considered as markers potentially related to blast resistant genes (Table 2).

In case of OPA-05 (5'AGGGGTGTTG3'), band sizing 1000 and 1200 bp were observed in 22 rice resistant genotypes. Resistant genotypes (IR-74886-55-2-3-2, IR-74053-144-2-3 and BG-379-2) did not show the presence of 1000 and 1200 bp marker bands. These bands were altogether absent in all susceptible rice genotypes (Figure 1 and Table 2).

The size of the amplified products of OPG-19 (5'GTCAGGGCAA3') range between 80 to 1400 bp. Out of 25, 20 rice resistant genotypes showed the presence of 500 bp marker band, which is supposed to be linked with blast resistance. Resistant genotypes IR-71527-44-1-1, IR-80922, Tarori, Pusa Basmati-1121 and Super Basmati did not show the presence of marker band (Figure 1; Tables 1 and 2). The amplified products of OPH-18 (5'GAATCGGCCA3') varied between 40 to 950 bp. Most of the bands were found to be monomorphic in nature, except bands with sizes 100 and 500 bp, observed only in 18 resistant rice genotypes (Figure 2; Tables 2 and 3).

Two blast resistance SCAR markers (P-265-550 and P-286-350) designed by Zhaung et al. (1998) were also verified in the current study. Instead of a single band as reported by Sandhu et al. (2003), many polymorphic bands with both SCAR primers were observed (Tables 2 and 3). A single, distinct and brightly resolved band of 900 bp size (in case of P-285-550) was observed only in 13 resistant rice genotypes; however, no such amplification was observed in susceptible rice genotypes (Figures 3 and 4; Tables 2 and 3).

Comparing resistance and susceptibility of the different fragments, P-265-550 (850 bp) and P-286-350 (900 bp) were identified as markers potentially related to the blast resistance gene in the resistant genotypes (Tables 2 and 3).

These findings suggest that the SCAR markers designed by Zhuang et al. (1998) and Naqvi and Chattoo (1996) are not universal RAPD/SCAR markers for all blast resistant rice genotypes. The availability of co-dominant RAPD markers for other blast resistance genes would be extremely useful in gene pyramiding studies and in the detailed mapping of loci for positional cloning projects, as well as being very useful in breeding programs for blast resistance rice. In almost all resistant genotypes, marker bands were not uniformly present. This was an indication that marker bands (either RAPD or SCAR) are supposed to be linked with different resistant genes and resistant genotypes are non-allelic in nature. Since, immunity against this disease is not known, enhancement of resistance is being sought by using resistant × resistant crosses. However, this gene pyra-

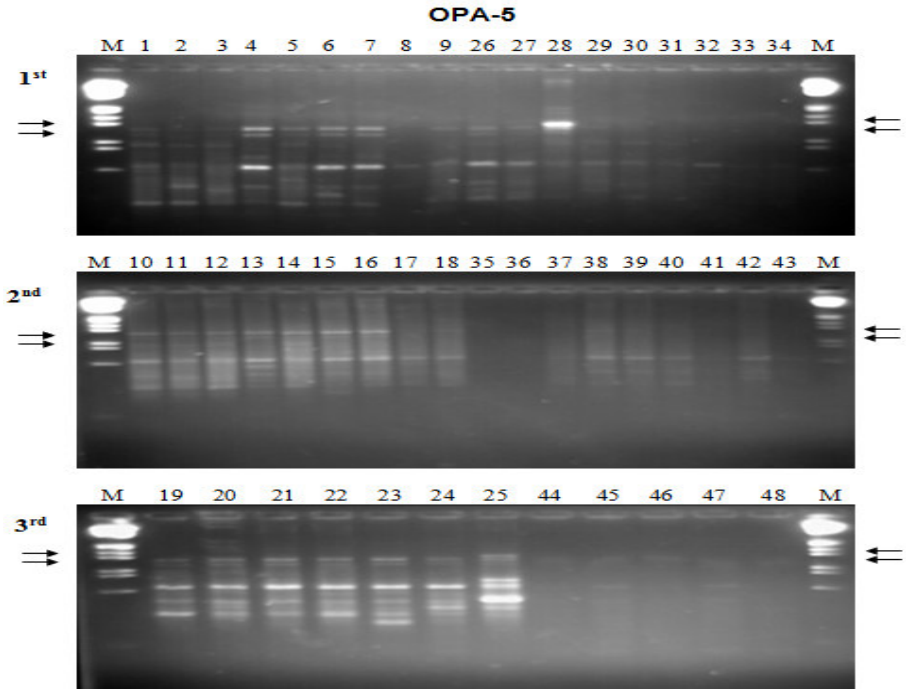

Figure 1. RAPD profile of 48 rice genotypes (Resistant genotypes: Lanes 1 to 9 in 1st gel,10 to 18 in 2nd gel and 19 to 25 in 3rd gel. Susceptible genotypes: Lanes 26 to 34 in 1st gel, 35 to 43 in 2nd gel and 44 to 48 in 3rd gel). M is the molecular marker DNA/*Eco*RI+Hind-III. Arrows display the polymorphic bands (1000 to 1200 bp) present in resistant genotypes.

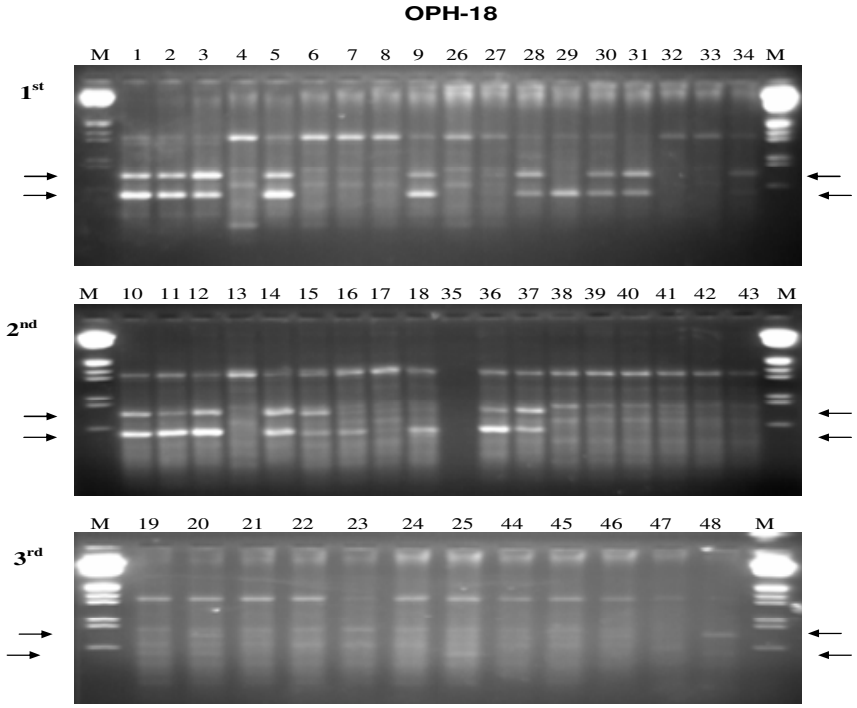

Figure 2. RAPD profile of 48 rice genotypes (resistant genotypes; 1 to 9 in 1st gel,10 to 18 in 2nd gel and 19 to 25 in 3rd gel; susceptible genotypes; 26 to 34 in 1st gel, 35 to 43 in 2nd gel and 44 to 48 in 3rd gel). M is the molecular marker DNA/*Eco*RI+Hind-III. Arrows display the polymorphic bands (100 and 500 bp) present in resistant genotypes.

Table 2. A comparative analysis of 10 random primers and 2 SCAR primers size of their amplified products and size of polymorphic bands present in resistant and susceptible genotypes.

Primers	Primers sequences	Size of amplified product (bp)		Polymorphic bands	
		Resistant genotypes	Susceptible genotypes	Resistant genotypes	Susceptible genotypes
RAPD primers					
OPA 5	5'AGGGGTGTTG3'	40 bp -1.2 kb	40 bp - 1.9 kb	1 and 1.2 kb	---
OPF 6	5'GGGAATTCGG3'	40 bp - 4.2 kb	40 bp - 4.2 kb	4000 bp	----
OPF 9	5'CCAAGCTTCC3'	70 -1000 bp	70 bp -947 bp	600 bp	----
OPF 17	5'AACCCGGGAA3'	90 -1300 bp	----	700 bp	----
OPF19	5'CCTCTAGACC3'	------	-----	------	------
OPG 17	5'ACGACCGACA3'	40 - 1600 bp	40 - 1200 bp	100 bp	-----
OPG 18	5'GGCTCATGTG3'	80 - 850 bp	30 - 900 bp	550 bp	----
OPG 19	5'GTCAGGGCAA3'	80 - 1400 bp	80 - 1400 bp	500 bp	---
OPH 18	5'GAATCGGCCA3'	40 - 950 bp	40 – 950 bp	100 and 500 bp	----
OPK 12	5'TGGCCCTCAC3'	70 - 947 bp	70 - 2000 bp	900bp	----
SCAR primers					
P 265-550(F)(R)	5'CAGCTGTTCAGTCGTTTG3' 5'CAGCTGTTCATACAAGAAAT3'	45 bp -1 kb	40 bp – 1 kb	850 bp	-----
P286-350 (F)(R)	5'GCTCCGCATTAACGGGAAG3' 5'AGCCGGCTCCGGAGGTGA3'	80 - 900 bp	80 - 900 bp	900 bp	------

Table 3. A comparative study of disease reaction observed at field level and presence of marker bands with eight random primers and two SCAR primers.

Genotypes	Reaction at field level				Reaction with RAPD primers										Reaction with SCAR primers	
	R	MR	MS	S	OPA 5	OPF 6	OPF 9	OPF 17	OPF 19	OPG 17	OPG18	OPG19	OPH18	OPK 12	P-265 550	P-286-350
IR-72107-4-159-1-3-3-3	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+
IR-74886-55-2-3-2	-	+	-	-	-	+	+	+	-	+	-	+	+	+	+	-
IR-74371-70-11	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+
PSBRC-80-1	+	-	-	-	+	+	+	-	-	-	+	+	-	+	+	+
IR-75298-59-3-1-3	+	-	-	-	+	+	+	+	-	+	-	+	+	-	+	+
IR-7388-1-2-7	-	+	-	-	+	-	+	-	-	-	+	+	+	-	+	+
IR-71604-4-1-4-7-10-2-1-3	-	+	-	-	+	+	+	-	-	-	+	+	-	-	+	+
IR-71527-44-1-1	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-
BW-391	+	-	-	-	+	+	+	+	-	+	-	+	+	+	+	-
IR-80920	+	-	-	-	+	+	+	+	-	+	+	-	+	-	+	-
IR-80922	+	-	-	-	+	+	+	-	-	+	-	+	+	-	-	-
IR-71700-247-1-1-2	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	+
IR-74374-46-1-1	+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	+
Vallabh-21	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	+
Basmati-370	-	-	+	-	+	+	+	-	-	+	+	+	+	-	+	-
Sugandha-4(APEDA)	-	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-
SVT-16	-	+	-	-	-	+	+	-	-	+	-	+	-	+	-	-
Tarori	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-	-
Tarori (APEDA)	-	+	-	-	+	+	-	-	-	-	-	+	+	-	-	-
SVT-28	-	-	+	-	+	+	-	-	-	-	-	+	+	-	-	-

Table 3. cont.

Vallabh Basmati-21	-	-	+	-	+	+	-	-	-	+	-	+	+	-	-	-
P-79	-	+	-	-	+	-	-	-	-	-	-	+	+	-	-	-
P-89	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-
Pusa-1121	-	+	-	-	+	+	+	-	-	+	-	-	+	-	+	+
Super Basmati(APEDA)	-	+	-	-	+	+	+	-	-	-	+	-	+	+	+	-
HKR-1	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
NDR-359	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
CSR-27	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
SVT-10	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
16-Macro	-	-	-	+	-	-		-	-	-	-	-	-	-	-	-
32-Macro	-		-	+	-	-	-	-	-	-	-	-	-	-	-	-
Tilakchandn	-	-	-	+	-	-	-	-	-	-	-	-	-		-	-
Sugandha-2	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Saket-4	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
39-Macro	-	-	-	+	-	-		-	-	-	-	-	-	-	-	-
23-Macro	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
SVT-5	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
SVT-21	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
NDR-180	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
P-136	-	-	-	+	-	-	-	-	-	-	-	-	-		-	-
MAUU-15B	-	-	-	+	-	-		-	-	-	-	-	-	-		-
NDR-18	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Ajay*PB-1	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
P-83	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
P-111	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
P-31	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Sathi	-	-	-	+		-	-	-	-	-	-	-	-	-	-	-
P-70	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

Symbol indication as: + = presence of marker band; - = absence of marker band; R = resistant genotypes (showing disease severity (%) less than 45% and AUDPC less than 1000); MR= moderate resistance genotypes (1000 - 1500); MS = moderate susceptible genotypes (1500 - 2000); and S = susceptible genotypes (more than 2000).

miding would be facilitated, if knowledge about markers linked with diverse blast resistance genes is made known to rice breeders.

The presence of molecular markers OPA-05, OPF-06, OPF-09, OPG-17, and OPK-12 in most resistant genotypes and the presence of molecular markers OPF-09 and OPF-19 only in other resistant genotypes revealed that resistance sources are non-allelic for resistance genes indicating the possibility of obtaining transgressive sergeants if crosses are made between these resistant genotypes. High levels of resistance under artificial epiphytotic conditions and the presence of marker bands with at least 4 - 5 random primers and SCAR primers tested in the screening of rice genotypes indicate the presence of many minor genes (Table 2). However, genotypes showed moderate level of resistance to the blast pathogen (IR-74886-55-2-3-2, IR-7388-1-2-7, IR-71604-4-1-4-7-10-2-1-3, Sugandha-4(APEDA), SVT-16, Tarori (APEDA), P-79, Pusa-1121, Super Basmati; markers that appeared with 2 - 3 random primers clearly indicate that these markers are linked with diverse resistance genes. The appearance of molecular markers ($OPA\text{-}05_{1000bp}$ and $OPA\text{-}05_{1200bp}$) in 20 resistant genotypes is an indication of commonality of genes controlling resistance to the blast pathogen. Presence of common marker bands in most of the resistant genotypes was an indication that at least one or two common resistance genes are present in all the resistant genotypes. The genotypes showing presence of marker bands with maximum number of random primers and SCAR primers have been selected for further testing and use in breeding programs. None of the susceptible genotype which showed the presence of RAPD/SCAR markers linked to blast resistance was an indication of tight linkage between RAPD/ SCAR markers and resistance gene(s).

Thus, desirable changes can be accomplished by following intensive crossing programmes between non-allelic parents and gene pyramiding of resistant genes through marker assisted selection (MAS).

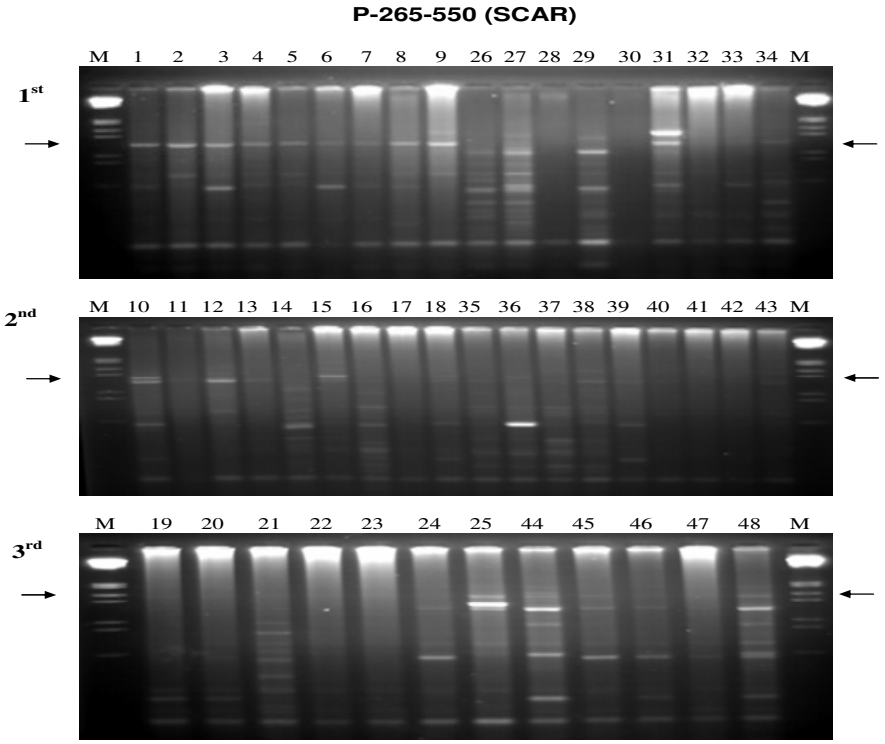

Figure 3. SCAR profile of 48 rice genotypes (resistant genotypes; 1 to 9 in 1st gel,10 to 18 in 2nd gel and 19 to 25 in 3rd gel; susceptible genotypes; 26 to 34 in 1st gel, 35 to 43 in 2nd gel and 44 to 48 in 3rd gel). M is the molecular marker DNA/*Eco*RI+Hind-III. Arrow displays the polymorphic band (850bp) present in resistant genotypes.

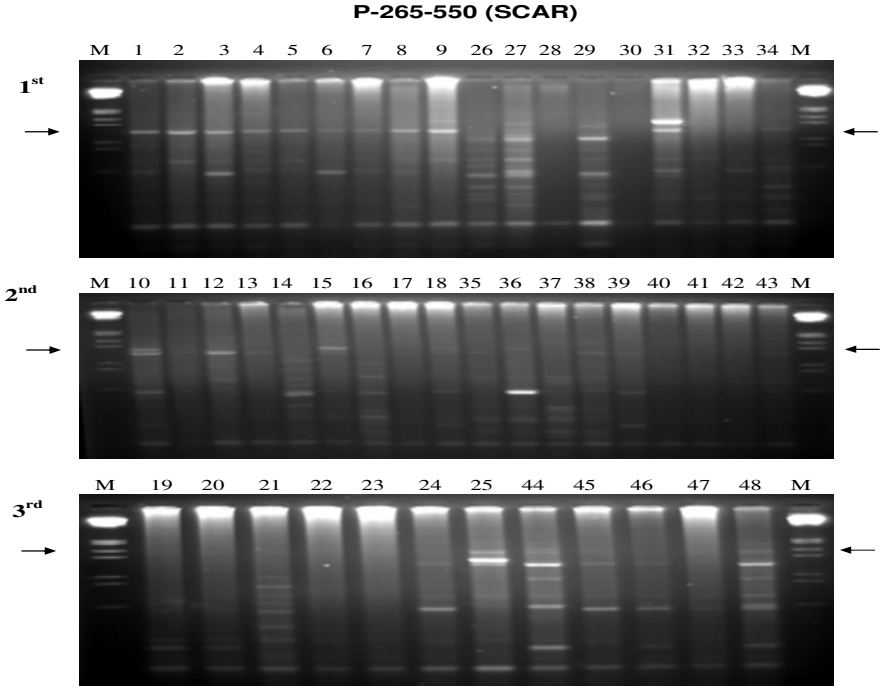

Figure 4. SCAR profile of 48 rice genotypes (resistant genotypes; 1 to 9 in 1st gel,10 to 18 in 2nd gel and 19 to 25 in 3rd gel; susceptible genotypes; 26 to 34 in 1st gel, 35 to 43 in 2nd gel and 44 to 48 in 3rd gel). M is the molecular marker DNA/*Eco*RI+Hind-III. Arrow displays the polymorphic band (850 bp), present in resistant genotypes.

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