African Journal of Biotechnology Vol. 10(17), pp. 3301-3321, 25 April, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2573
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Identification of Microsatellite (SSR) and RAPD Markers Linked to Rice Blast Disease Resistance gene in Rice (*Oryza sativa* L.)

Immanuel Selvaraj C[1,2]*, Pothiraj Nagarajan[2], Thiyagarajan K[3], Bharathi M[4] and Rabindran R[5]

[1]School of Biosciences and Technology, Plant Biotechnology Division, Vellore Institute of Technology University. Vellore, India.
[2]Center for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, India. Vellore, India.
[3]Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India.
[4]Centre for Plant Protection Studies (Entomology), Tamil Nadu Agricultural University, Coimbatore, India.
[5]Centre for Plant Protection Studies (Plant Pathology), Tamil Nadu Agricultural University, Coimbatore, India.

Accepted 21 March, 2011

A mapping population comprising 220 F_2 individuals was developed by raising the selfed F_1 seed of a single hybrid plant, White Ponni x Moroberekan. Two hundred and twenty two F_3 families were phenotyped for blast resistance under artificial condition. Fifty random amplified polymorphic (RAPD) primers were used to identify parental polymorphism. Twelve RAPD markers which showed clear polymorphism were scored in the F_2 mapping population. Out of 12 markers, 11 loci (91.97 %) fitted into the expected segregation ratio of 3:1 based on χ^2 - test at 0.05% probability value. Four hundred microsatellite primer pairs were used in the parental survey, Out of which 52 simple sequence repeats (SSR) primers were polymorphic. Out of 52 marker loci, 44 marker loci (84.61%) fitted into the expected Mendelian segregation ratio of 1:2:1 based on χ^2 - test at 0.05% probability value. The multipoint analysis resulted in the construction of preliminary genetic linkage map with 40 loci. One way analysis of variance (ANOVA) was performed to identify the marker phenotype association which resulted in the identification of 23 SSR markers putatively associated for the six traits studied. Three SSR markers (RM 5757, RM 451 and RM 492 from chromosomes four and two) were linked for leaf blast resistance, six markers for days to flowering, nine markers for pigmentation trait, two markers for plant height, four markers for panicle length and seven markers linked for grain yield per plant. Out of 12 RAPD markers studied, 7 were found to be linked to three traits viz., leaf blast resistance (OPBB 5_{258}, OPBB 5_{194}, OPAL 16_{940} and OPBD 12_{680}), five markers for glume purple tip (GPT) and one marker for grain yield per plant based on single marker analysis.

Key words: *Magnaporthe grisea* (Hebert) Barr, *Oryza sativa*. L., marker assisted selection (MAS).

INTRODUCTION

The rice blast disease is caused by the fungus *Pyricularia grisea*, which, in its sexual state, is known as *Magnaporthe grisea* (Hebert) Barr. (anamorphe: *Pyricularia grisea* Cav.), a filamentous heterothallic ascomycoteous fungus. Rice blast is one of the most devastating diseases of rice and often reduces rice yields greatly in rice-growing countries under disease-conducive conditions. The disease can strike all aerial parts of the plant. Most infec-tions occur on the leaves, causing diamond-shaped lesions with a grey or white centre to appear, or on the panicles, which turn white and die before being filled with grain. To date, more than 40 major blast resistance genes have been mapped (Tabien et al., 2000; Fukuoka and Okuno, 2001; Sallaud et al., 2003). However, some of these genes could be identical or allelic, since very few allelism tests were performed. Kiyosawa (1984) described differential

*Corresponding author. Email: immmer@gmail.com or imm_mer2003@yahoo.co.uk. Tel: +91 416 2202546 or +91 98654 20103. Fax: +91 416 2243092.

cultivars with one or two resistance genes named *Pi-*, followed by a different letter for each gene. Some of these genes have several alleles, such as loci *Pi-z, Pi-ta* with two alleles and *Pi-k* with five alleles. It has been proposed that *Pi3-(t)* is allelic to *Pi-5(t),* as both confer similar resistance spectra to a variety of blast lineages. Liu et al. (2002) demonstrated that the resistance genes *Pi-9(t)* and *Pi-2(t)* were tightly linked and allelic.

Several blast resistant varieties have been and are being released regularly. They lose resistance due to the change in the race pattern of the blast fungus. Fungicides fail to control blast remarkably as it is too static to deal with the dynamic relationships between plants and disease that are deeply tied to the surrounding ecology. Resistance is considered durable when it remains effective in a cultivar despite widespread cultivation in an environment favoring the disease.

In different patho-systems, durable resistance is variously controlled by single genes, multiple genes with cumulative effects and polygenes. The resistance may be either complete or incomplete (Parlevliet, 1988). Some upland cultivars such as the traditional African cultivars, Moroberekan and OS6, have been cultivated for many years in large areas in West Africa without high losses of blast. Five resistance genes have been identified in African cultivar Moroberekan. These cultivars have been widely used as resistance donors in breeding programs.

Molecular markers are useful tools for monitoring gene introgressions and to detect polymorphism among species. The use of molecular markers can help in estimating the overall genetic variability, visualize the proportion of the genome introgressed from the donor, identify the genes related to the increase in the phenotypic value of analyzed traits, and then allow marker assisted selection in subsequent generations of these introgression lines (Brondani et al., 2003).

However, the presence or absence of the associated molecular marker could indicate at a very early stage, the presence or absence of the desired target gene. Selection based on genetic information was retrieved through the application of molecular markers. Marker assisted selection (MAS) could be employed to enhance plant breeding efforts and to speed up the creation of new cultivars. A molecular marker very closely linked to the target gene can act as a tag, which can be used for indirect selection of the gene(s) in a breeding programme. This process is referred to as MAS in breeding (Witcombe and Hash, 2000; Asins, 2002; Liu et al., 2004; Zhang et al., 2005). In this paper, attempts were made to reveal the random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers linked to rice blast disease resistance by single marker analysis (SMA)

MATERIALS AND METHODS

Hybridization program

Crossing was effected with the leaf blast resistant cultivar, Moroberekan, as a pollen parent and the susceptible cultivar, White Ponni as a ovule parent. Moroberekan (A West African cultivar) is known for its durable resistance to rice blast disease (Wang et al., 1994) and the susceptible variety, White Ponni (selection from the cross derivative, Taichung 65/2 x Mayang Ebos - 80) is known for its fine grain quality and as a moderate yielder (45 quintals per hectare). The genotypes were raised in nursery beds with three staggered sowings at 10 to 15 days interval to ensure synchronized flowering to enable hybridization.

Development of mapping population

The crossed seeds thus obtained were raised in the field and selfed. The trueness of the hybrids were confirmed both by morphological and molecular markers. A total of 220 selfed seeds of an identified F_1 plant were collected and raised as F_2s by single seed descent method to generate segregating population. Leaves were collected from all the 220 F_2 individuals for DNA extraction.

Biometric observations recorded

In the segregating population (White Ponni / Moroberekan), the following biometric observations viz., days to 50% flowering, plant height, presence or absence of pigmentation at the tip of the spikelets / Glume (GPT) / culm region, number of tillers per plant, number of productive tillers per plant, panicle length and grain yield per plant were recorded.

Artificial screening for leaf blast disease reaction in parents and F_3 families

Artificial screening for rice blast disease was done in the year 2005, in Paddy Breeding Station, Coimbatore, Tamilnadu, in a specially constructed screen house with good irrigation facilities fitted with mist blowers, which can spray water in a fine mist inside the chamber. Subsequently, the seedlings were misted 4 to 5 times at intervals. The screen house was maintained at 32 to 37°C (day temperature) and 94 to 96% relative humidity (RH) for the potential disease occurrence. The rate of sporulation increases with increase in relative humidity provided with lower night temperature with minimum of 25°C. Inoculations with *M. grisea* Hebert (Barr) were performed 3 weeks after sowing by spraying with conidial suspensions. For artificial rice blast screening, the method by Sallaud et al. (2003) was followed. Isolation of rice blast fungus was done from paddy breeding station, Coimbatore. Artificial culturing of the rice blast fungal inoculum is shown stepwisely in Figure 1. For the spray method, 30 ml of a 50,000 conidia.ml^{-1} suspension with 0.5% gelatin were sprayed on each tray. The observation on the disease incidence was recorded, when the susceptible check was severely infected by blast. Observations were recorded from 20 plants in each F_3 family following Standard Evaluation System (SES, 2002) on 0-9 scale at 25th day after sowing. The resistant check used was IR 64. Observations were recorded in plants, when they were at third leaf stage. The screen house, disease symptoms and variations of occurrence of the disease in incidence in F_3 population is shown in Figure 2. The grade and criterion based on standard evaluation system is as follows score 0 - no lesions observed; score 1 - small brown specks of pin point size or larger brown specks without sporulating centre; score 3 - small roundish to slightly elongated necrotic grey sporulating spots about 1 to 2 mm in diameter with a distinct brown margin; score 5 – narrow or slight elliptical lesions, 1 to 2 mm in breadth, more than 3 mm long with brown margin; score 7 - broad spindle shaped lesion with yellow, brown or purple margin; score 9 - rapidly coalescing small, whitish, greyish or bluish lesions without distinct margins.

The potential disease incidence percent (PDI %) was worked out

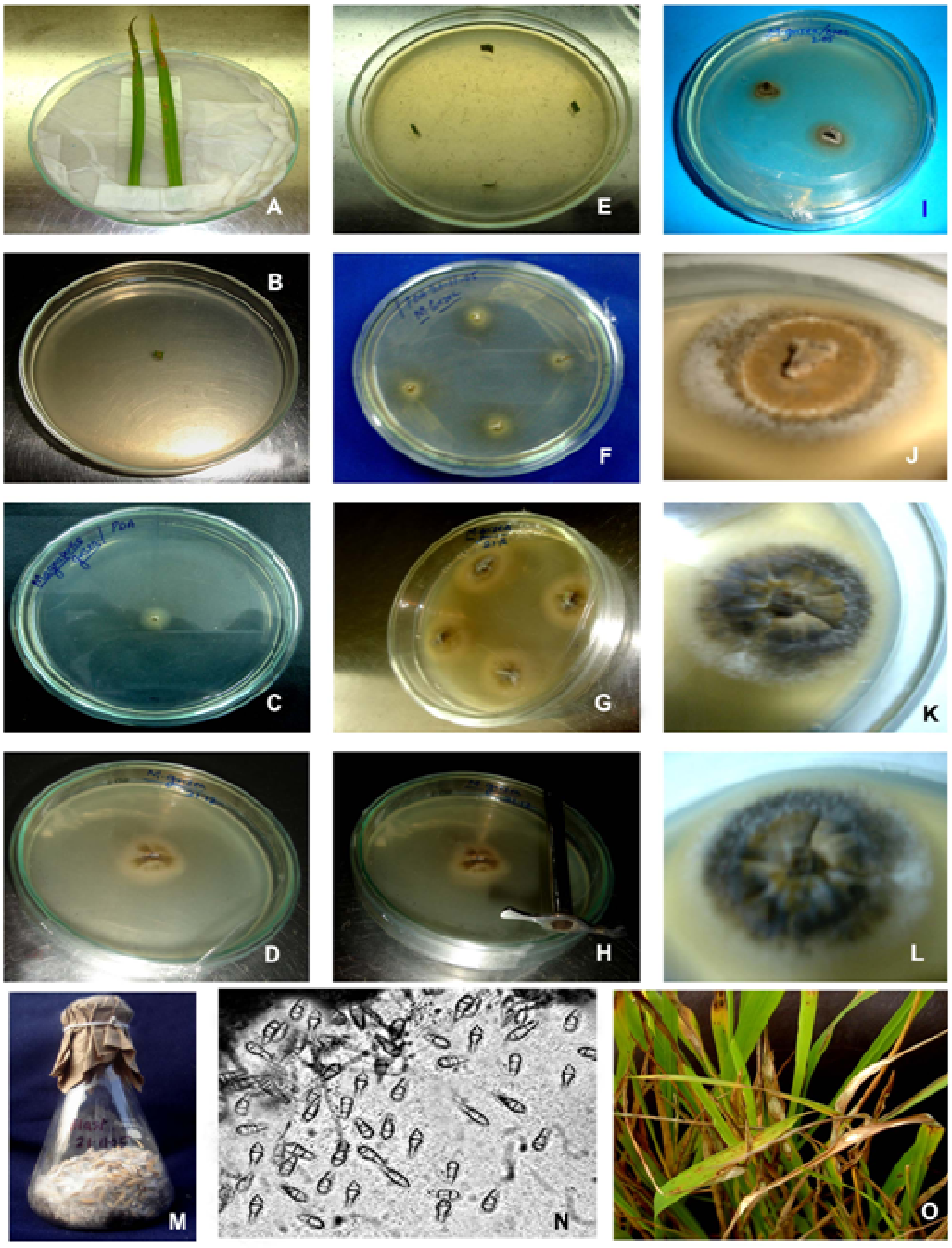

Figure 1. Isolation and sporulation of rice blast fungus (*M. grisea*) for artificial disease screening. A, Collection of typical, young, greenish rice blast symptom from 15 days old rice seedling in a moist chamber; B and E, after surface sterilization with 0.1% $HgCl_2$, the symptom alone is half cut and placed in PDA or OMA medium; C and F, five days after inoculation of the symptom, kept at 24 to 26°C, immersed mycelial growth appears; D and G, eleven days after inoculation, kept at 24 to 26°C, individual colony with pale white mycelial growth appears; H, subculturing was done after eleventh day up to 3 to 4 subcultures from the growing mycelial tips. J and K, an effused colony, thinly hairy, turning from olivaceous brown to greyish brown with immersed mycelium and wedge shaped centre was seen. L, full grown rice blast (*M. oryzae*) colony with typical colony morphology; M, Inoculated conical flasks maintained at 27°C at 90% RH inside fluorescent incubator for 15 days; N, observation of rice blast spores at 15 - 20 days after inoculation in Leitz (Flovert FS) microscope under the magnification of 100x. O, disease incidence after spraying of artificial rice blast sporulated inoculum with spore concentration adjusted to 50,000 conidia.ml^{-1} suspension with 0.5% gelatin spores (approximately) 2 to 3 times inside the screen house for disease induction.

using the formula of McKinney (1923): Sum of numerical rating / number of leaves observed x 100 / maximum disease grade.

Isolation of DNA

Fresh leaf samples collected from 15 days old seedlings of parental genotypes and the segregating population was used for isolation and purification of total genomic DNA following the method of McCouch et al. (1988). A total of 50 decamer primers supplied by Operon Technologies Inc., Alameda, California, USA were used in the study for parental genotyping. Seven RAPD primers which are highly polymorphic were used to genotype the F_2 mapping population which produced 12 polymorphic markers namely, OPAL 16_{940}, OPAL 18_{1054}, OPBA 15_{458}, OPBA 15_{742}, OPBD 12_{680}, OPBD 12_{600}, OPBD 12_{1584}, OPM 9_{350}, OPBB 5_{194}, OPBB 5_{258}, OPBB 5_{700} and OPBB 13_{755}. The details of RAPD markers along with the sequences are given in Table 1.

RAPD analysis

The basic procedure of the RAPD reaction was as follows: 1x reaction buffer, 0.1 mM of each dNTP, Mg^{2+} 2 mM, *Taq* polymerase 1.25 units, template DNA 20 ng, primer 25 ng, each reaction volume is made up to 25 µl with nuclease water. The amplification reactions were carried out by the following profile: 94°C, 5 min for one cycle; 94°C, 1 min; 37°C, 1 min; 72°C, 2 min for 40 cycles; then 72°C, 5 min. The amplification products were analyzed by electrophoresis in 1.5% agarose gels with ethidium bromide. The results were visualized and documented in a gel documentation system (Alpha Imager ^{TM}1200, Alpha Innotech Corp., California, USA).

Microsatellite marker analysis

Microsatellite marker analysis was done based on the protocol reported by McCouch et al. (2002). A set of 400 SSR primers from Research Genetics Inc., USA and from M/s Sigma Aldrich Inc., USA was used for PCR amplification using DNA from parents to identify polymorphic primers. The polymorphic primers were surveyed on the individuals of F_2 population. PCR amplifications were performed in 96-well plates on thermal cycler PTC-100^{TM} MJ Research Inc., USA (or) Eppendorf gradient thermal cycler. The PCR reaction was done in volumes of 15 µl containing 20 ng of DNA, 0.2 µM of each forward and reverse primers, 100 µM of dNTPs, 10 mm Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin and 0.5 unit of *Taq* DNA polymerase. The general polymerase chain reaction (PCR) profile is as follows: 94°C for 5 min followed by 35 cycles of 1 min at 94°C, 1 min at 56 to 67°C, specific to the primer pairs used and 1 min at 72°C with final extension of 5 min at 72 and 4°C for infinity to hold the sample. The patterns of amplified products were resolved by silver staining following the procedure described by Panaud et al. (1996). The details of SSR primers used in this study are listed in Table 2.

Data analysis for the F_2 mapping population

The basic statistics viz., mean, standard deviation and coefficient of variation were worked out for the six quantitative traits along with disease reaction and pigmentation trait. A standard analysis of variance (one way ANOVA) was used to evaluate mean differences among the three classes defined by each SSR marker locus and two classes defined by RAPD markers for different quantitative traits to establish phenotype–marker association as suggested byBeckmann and Soller (1986) and Edwards et al. (1987) using the general linear model (GLM) procedure of the Statistical Analysis System (SAS) program (SAS Institute Inc., 1990). A significant F-test ($P < 0.01$) indicated segregation of marker locus genotypic classes with phenotype. Significance at the $P = 0.05$ level was considered suggestive of a quantitative trait loci (QTL) at the marker locus. Simple regression analysis was also performed according to the method described by Haley and Knott (1992) using regression coefficient as a function of unknown QTL parameters. Then, stepwise regression was done to identify the most probable marker combinations associated with the trait of interest with their contributions towards total phenotypic variance. Each quantitative trait was treated as dependent variable and various marker genotypes (scored 1, 2 and 3 for SSR and 0 and 1 for RAPD) as independent variables.

Segregation ratios for the SSR and RAPD marker classes were tested for the expected 1:2:1 and 3:1, respectively with chi-square test. Multi-point analysis was carried out to confirm the order of the SSR markers and construct a primary map of White Ponni /Moroberekan using MAPMAKER / EXP MS-DOS 3.0. The Kosambi mapping function was used in the analysis (Kosambi, 1944).

RESULTS

Phenotypic traits and their variations in F_2 mapping population

Out of five phenotypic traits recorded in the 220 individual plants of the mapping population, parents showed a high level of variation for pigmentation, days to flowering, plant height (Figure 3), panicle length and grain yield per plant. White Ponni recorded phenotypic values higher than Moroberekan for days to flowering, plant height, panicle length and grain yield per plant. The genotype Moroberekan possessed the glume pigmentation trait (GPT) character, whereas White Ponni lacked it. The frequency distribution of quantitative traits in F_2 population recorded is illustrated in the histogram in Figure 4. The GPT trait was recorded in the F_2 mapping population depicted in Figure 5.

The F_2 families exhibited a wider variation from parents for all the traits studied. Wider variation among the F_2 families for plant height was observed as it ranged from 48.00 to 125 cm with a mean height of 81.66 cm. Days to flowering among the F_2 individuals ranged from 71 to 126 days with a mean of 99.78 days. Panicle length deviated from 11.00 to 27.00 cm with the mean length of 20.59 cm. Grain yield per plant ranged from 19.54 to 100.26 g with a mean yield of 45.81 g. Phenotypic values of some of the traits studied in the F_2 population exceeded the parental values indicating the presence of transgressive segregation in all the traits. The mean, standard deviation and coefficient of variation for the four quantitative traits viz., plant height, days to flowering, panicle length, grain yield per plant and a qualitative trait viz., purple pigmentation (GPT) trait are given in Table 3.

Rice blast screening of the 220 F_3 families

Two hundred and twenty F_3 families (Twenty plants from each of the corresponding 220 F_2 individual plants) were

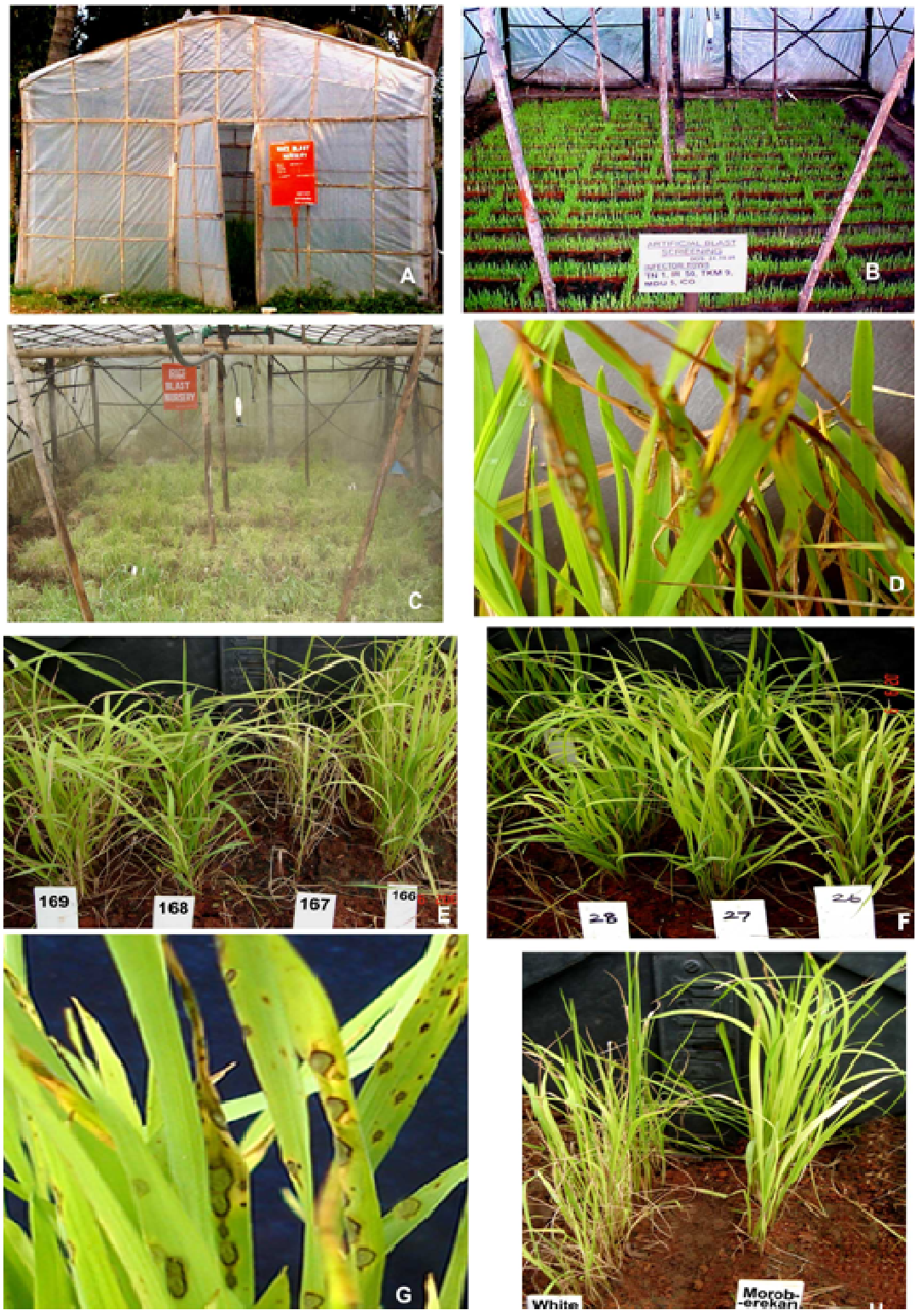

Figure 2. Experimental setup for artificial rice blast disease screening and variations of disease incidence observed in F_3 population and parents. A and B, Well equipped artificial screen houses for rice blast screening with prepared trays beds; C, artificial screen house equipped with mist blowers to maintain RH at 95% and leaf wetness; D, incidence of rice blast disease indicated by specks and burnt up appearance, inside the screen house; E, variations observed in the degree of infection within the F3 families namely, 169, 167- susceptible; 168- moderately resistant; 166- moderately susceptible; F, resistant F_3 families of the mapping population; G, closer view of rice leaf blast disease at earlier stage; H, highly susceptible parent, White ponni (P_1) and the resistant parent, Moroberekan (P_2).

Table 1. Details of polymorphic RAPD markers used in segregating F_2 mapping population.

S/N	RAPD marker	Primer sequence (5'-3')
1	OPAL 16_{940}	CTTTCGAGGG
2	OPAL 18_{1054}	GGAGTGGACT
3	OPBA 15_{458}	GAAGACCTGG
4	OPBA 15_{742}	GAAGACCTGG
5	OPBD 12_{680}	GGGAACCGTC
6	OPBD 12_{600}	GGGAACCGTC
7	OPBD 12_{1584}	GGGAACCGTC
8	OPM 9_{350}	GTCTTGCGGA
9	OPBB 5_{194}	GGGCCGAACA
10	OPBB 5_{258}	GGGCCGAACA
11	OPBB 5_{700}	GGGCCGAACA
12	OPBB 13_{755}	CTTCGGTGTG

subjected to artificial blast screening nursery. Out of 220 F_3 families, 110 were resistant (R) with the mean PDI percentage range of 12.96 to 33.33 and the mean disease reaction scores ranging from 1.17 to 3.00. Thirty four F_3 families were moderately resistant (MR) with the mean PDI percentage ranging from 33.51 to 55.19 and the mean disease scores ranging from 3.02 to 4.97. Twenty seven F_3 families were moderately susceptible (MS) with the mean PDI percent ranging from 56.67 to 77.41 and 5.10 to 6.97. Forty nine F_3 families were highly susceptible (S) with the mean PDI percent ranging from 79.26 to 99.99 and the mean scores ranging from 7.13 to 9.00 (Figure 6). The mean PDI percentage and the mean disease scores recorded in the F_3 families and parents are given in Table 4.

Mapping survey of the F_2 segregating population by RAPD markers

A total of 50 RAPD primers were surveyed on parents to identify polymorphic markers between them. Seven RAPD primers which showed distinct polymorphism were used to genotype the F_2 mapping population which produced 12 markers namely, OPAL 16_{940}, OPAL 18_{1054}, OPBA 15_{458}, OPBA 15_{742}, OPBD 12_{680}, OPBD 12_{600}, OPBD 12_{1584}, OPM 9_{350}, OPBB 5_{194}, OPBB 5_{258}, OPBB 5_{700} and OPBB13_{755}. The banding pattern of F_2 individuals for the primer OPBD 12 is shown in Figure 7. F_2 analysis results indicated that, out of 12 markers, 11 fit to the expected Mendelian segregation ratio of 3:1 and the remaining marker deviated from the Mendelian segregation (Table 5).

Mapping survey using micro-satellite markers

A subset of 57 SSR primers from a total of 400 polymorphic primer pairs was surveyed on 220 F_2 individuals to establish their segregation pattern. Out of 52 primer pairs used, the amplified products of 5 primers could not be scored. Only 52 SSR primer pairs produced unambiguous amplified products. Maximum number of markers were scored on chromosome 7 (10 primers) followed by chromosome 1 (9 primers). Multiple copy fragments were observed in some of the primers. Only a single polymorphic fragment was scored among the multiple fragments. The segregation pattern of SSR marker RM 451 in the F_2 individuals is shown in Figure 8. Out of 52 marker loci, 44 (84.61%) fit into the expected segregation ratio of 1:2:1 based on χ^2 test at 0.05% probability value (Table 6). Out of 8 markers which deviated from the Mendelian segregation ratio, 5 (9.61%) exhibited segregation distortion towards Moroberekan and 3 (5.77%) skewed towards White Ponni. The overall allele frequency for the 44 loci showed an overabundance of heterozygotic alleles.

Construction of preliminary linkage map using SSR markers

A set of 52 SSR primer pairs which produced polymorphic phic markers between parents was surveyed on 220 F_2 individuals. The segregation data were used for preliminary genetic linkage map construction. A total of 12 markers remained unlinked. Out of these 12 marker loci, 9 (RM 168, RM 280, RM 411, RM 472, RM 492, RM 551, RM 566, RM 4584 and RM 5102) did not show significant deviation from the expected Mendelian segregation ratio indicating their independent assortment. The markers RM 566 and RM 2878, skewed towards White Ponni and the marker RM 81a skewed towards Moroberekan

When two-point analysis was done involving 52 SSR

Table 2. Details of micro-satellite primers used in the F_2 mapping population.

S/N	Primer name	Chromosome no.	Forward primer	Reverse primer
1	RM 84	1	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC
2	RM 472	1	CCATGGCCTGAGAGAGAG	AGCTAAATGGCCATACGGTG
3	RM 579	1	TCCGAGTGGTTATGCAAATG	AATTGTGTCCAATGGGCTGT
4	RM 582	1	TCTGTTGCCGATTTGTTCG	AAATGGCTTACCTGCTGTCTC
5	RM 6466	1	CGAACGAGAACTCCCTCATG	ATTGCACCAAGAGGAGATCG
6	RM 5638	1	GGCTTCCTCATCGCCATC	CTGAGCAGCATTCCAGTCTG
7	RM 8053	1	AGACATTGCCGATGATAGG	AAGTACCCCACCGAATAGAG
8	RM 8070	1	AAATGGACTCGCTCCTAAAC	AGGAGCGAATTTTATTGCTACT
9	RM 211	2	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG
10	RM 279	2	CCTCTCACTCACGTGGACTCC	CCTCACCCTAGGCTTTGATATGC
11	RM 492	2	CCAAAAATAGCGCGAGAGAG	AAGACGTACATGGGTCAGGC
12	RM 81a	3	GAGTGCTTGTGCAAGATCCA	CTTCTTCACTCATGCAGTTC
13	RM 168	3	GAAACGAATCAATCCACGGC	TGCTGCTTGCCTGCTTCCTTT
14	RM 411	3	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG
15	RM 545	3	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG
16	RM 1002	3	AGCATGGGGATTTAGGAACC	GAACCAGACAAGCAAAACGG
17	RM 3202	3	TTCACTTCCTATTGGCGGC	TCATCATCAGTCCAGCATCG
18	RM 280	4	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG
19	RM 451	4	GATCCCCTCCGTCAAACAC	CCCTTCTCCTTTCCTCAACC
20	RM 551	4	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG
21	RM 5757	4	CCTGAGACCATATGCTGCTG	GAGGGAGCATCATTAGCTGG
22	RM 122	5	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC
23	RM 574	5	GGCGAATTCTTTGCACTTGG	ACGGTTTGGTAGGGTGTCAC
24	RM 1237	5	CTCCGCGAGCTTTAGAAGAG	CACATACTCTGGCTCTCCCG
25	RM 402	6	GAGCCATGGAAAGATGCATG	TCAGCTGGCCTATGACAATG
26	RM 584	6	AGAAAGTGGATCAGGAAGGC	GATCCTGCAGGTAACCACAC
27	RM 585	6	CAGTCTTGCTCCGTTTGTTG	CTGTGACTGACTTGGTCATAGG
28	RM 1163	6	TCTAGGGTTAGGGTTTCGCC	AGGTCGGTTTCCTTTTGTCC
29	RM 70	7	GTGGACTTCATTTCAACTCG	GATGTATAAGATAGTCCC
30	RM 432	7	TTCTGTCTCACGCTGGATTG	AGCTGCGTACGTGATGAATG
31	RM 1048	7	AATTTTTAGTTTGGGGTAGA	CAAGCCTATAATGTGAATTG
32	RM 1279	7	GGGTATAAAATGCGTGGCAC	ATGGATGGTACGAGGACGAG
33	RM 2878	7	AGCAGAGCAATTTAACTAAG	GCCTATCATAATATCCATCA
34	RM 3186	7	CGACCAAGAGATGCTTCCTC	GAGTAGAAGGTGAGGCCACG
35	RM 3555	7	TGGAAGTTTCCTGGCGATAG	TGGTTGGACTGAAAAGTCCC
36	RM 3583	7	GGATGCCATGTCATCATCTG	TACAATTTGGCGACCTCCTC
37	RM 4584	7	CCATTTAAACATAGAAAAAC	CCTATTTAATATAGCACCAG
38	RM 5508	7	TCGCACACTAGCTCGATCAG	TCGCACACTAGCTCGATCAG
39	RM 80	8	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCACCG
40	RM 1309	8	CAGATCACAACCCTGCACTG	ATGGTTGCAACCTCTCCCTC
41	RM 4955	8	GCATCCAGCAATATAATCAA	CAAGGATTTTGTTAAGTGGG
42	RM 7285	8	GCGGCTATTGTAAGTGTTTG	TATATGAGTGCCACATGACG
43	RM 566	9	ACCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC
44	RM 5102	9	AATTTTCACCTACATTGTAA	AAGCATAGAAATGTTTGTAT
45	RM 216	10	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
46	RM 244	10	CCGACTGTTCGTCCTTATCA	CTGCTCTCGGGTGAACGT
47	RM 206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
48	RM 209	11	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC
49	RM 332	11	GCGAAGGCGAAGGTGAAG	CATGAGTGATCTCACTCACCC
50	RM 3331	12	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC
51	RM 4589	12	GTTTAAACATGGGAGGTGTC	CGAAATTTCTGAAATTTGGA
52	RM 5341	12	ATTTGATACATGGACGATGC	TGCATTTTCCATACAATACG

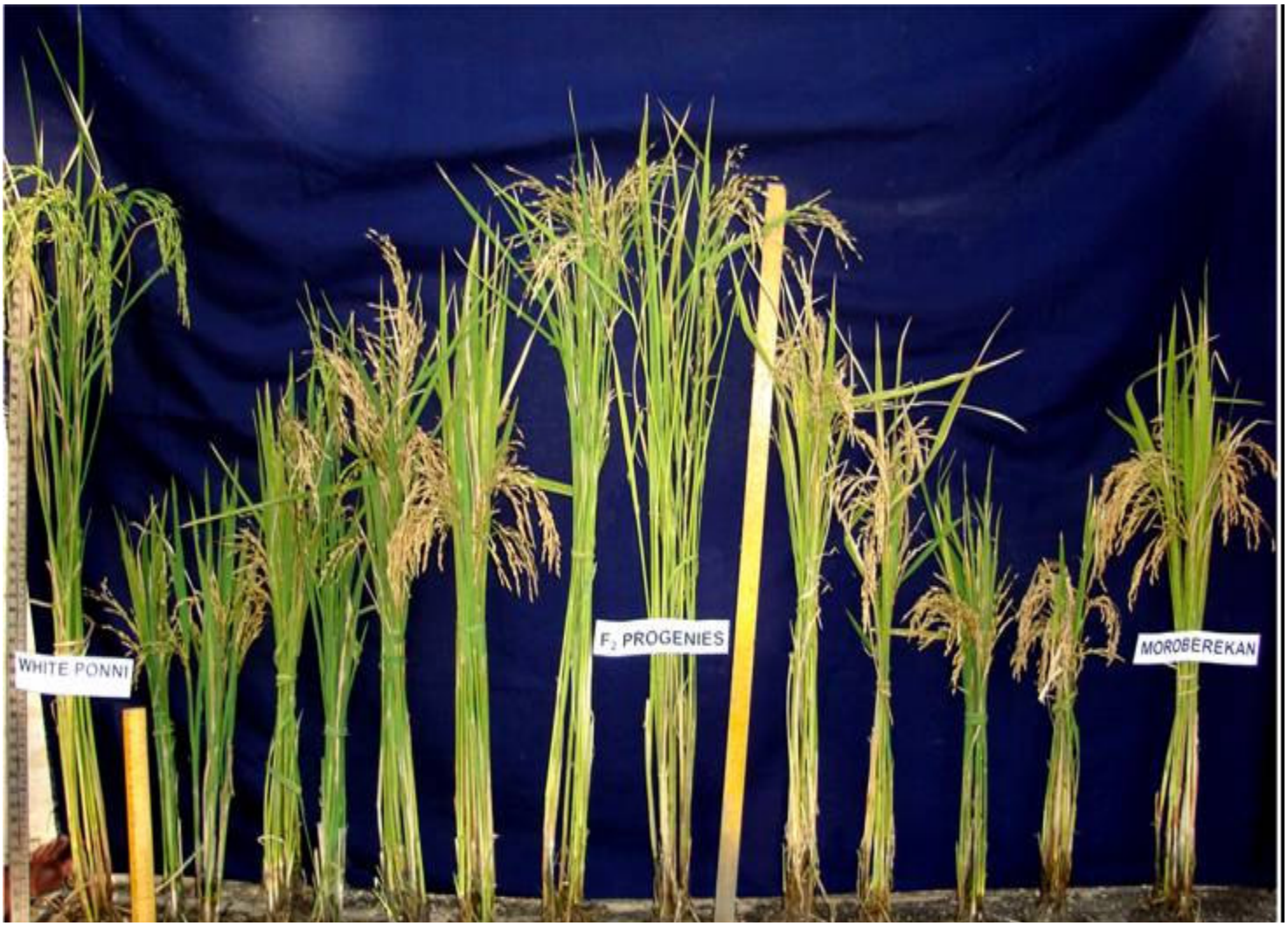

Figure 3. Raising F_2 mapping population and its progeny segregation (White Ponni x Moroberekan).

markers and 12 RAPD markers, 12 SSR markers remained unlinked. The RAPD markers linked with either SSR marker or with other RAPD markers. Out of 12 RAPD markers, only 3 (OPBD 12_{600}, OPBB 5_{258} and OPBB 5_{194}) were found to be linked with SSR markers and the remaining markers paired themselves. OPBD 12_{600} and OPBB 5_{194} was linked with RM1309 on chromosome 8 but these two RAPD markers paired between themselves making the situation complex to assign the RAPD markers on the specific chromosome. OPBB 5_{194} was linked with RM 7285 on chromosome 8 and OPBB 5_{258} was linked to RM1048 on chromosome 7. The other 9 RAPD markers linked themselves making the situation more complex to place the RAPD markers on specific chromosome. The multipoint analysis carried out on the segregating 52 marker loci using MAPMAKER program resulted in the construction of linkage map with 40 loci. The linkage map thus constructed involving 40 SSR loci is depicted in Figure 9.

Single marker analysis (SMA) to identify the RAPD and SSR markers linked to leaf blast resistance and other quantitative traits

One way ANOVA performed on the mean of groups formed based on the individual segregation pattern of 52 SSR marker loci resulted in the identification of 23 SSR markers putatively associated for the 5 quantitative traits and one qualitative trait studied. These markers were spread across the first nine chromosomes from 1 to 9. A simple regression analysis was also performed by keeping the phenotypic value as the dependent variable and the individual segregation pattern of SSR marker loci as independent variable.

A total of 31 linked markers were identified for 6 traits through SMA from the 23 SSR primers, in which some primers were linked for more than one traits studied. Among the 31 linked markers, 3 SSR markers were linked for leaf blast resistance, 6 markers were linked for

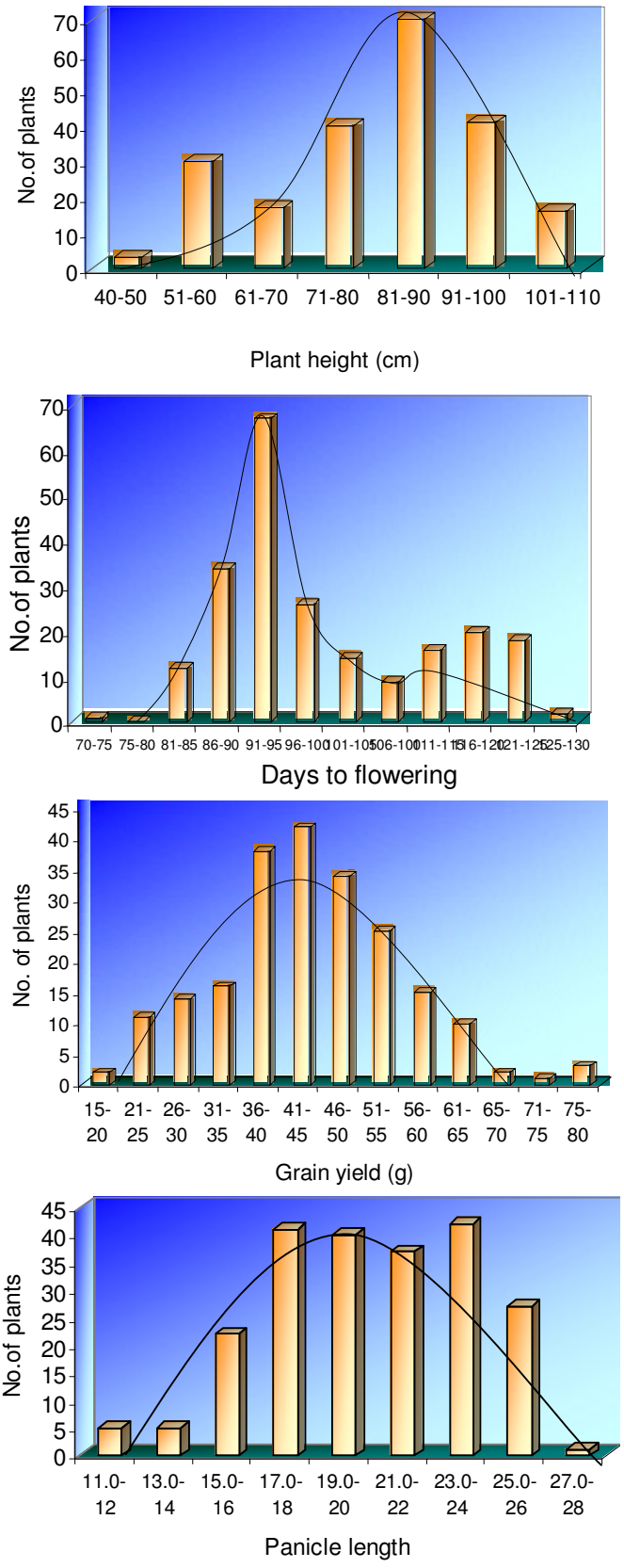

Figure 4. Frequency distribution of phenotypic traits over the F_2 populations of White Ponni / Moroberekan.

days to flowering, 9 markers were linked for pigmentation trait, 7 markers were linked for grain yield per plant, 2 markers were linked for plant height and 4 linked markers were for panicle length. Some of the SSR markers were linked for more than one trait in his study, viz., RM 451 (chromosome 4) (Figure 8) for leaf blast resistance and panicle length, RM 574 (chromosome 5) for days to flowering, plant height and grain yield per plant and RM 402, RM 584, RM 585, RM 1163 and RM 3202 (chromosome 6) were linked to both GPT (pigmentation) trait and days to flowering.

The details of the markers linked, the regression (R^2 per cent) values and the probability values are given in Table 7. Out of 12 RAPD markers studied, 7 markers were found to be linked to 3 traits viz., leaf blast resistance (OPAL 16_{940}, OPBD 12_{680}, OPBB 5_{258} and OPBB 5_{194}), pigmentation character / GPT (OPAL 16_{940}, OPBD 12_{680}, OPAL 18_{680}, OPBD 12_{600} and OPBB 13_{755}). Among the 7 markers, OPAL 16_{940} was linked to more than two traits namely, leaf blast resistance, pigmentation character / GPT and grain yield per plant (Table 7).

DISCUSSION

The rice blast disease caused by *M. grisea* (Hebert) Barr. (asexual form known as *P. grisea* (Cooke) Sacc.), is one of the most serious fungal disease which is widespread and threatening the world rice production (Yu et al., 1996). Numerous races of the fungus are prevalent. Blast resistance genes, commonly called *Pi* as genes, providing a broad spectrum of resistance against the most prevalent races can be extremely valuable in rice breeding efforts (Fjellstrom, 2003).

Choosing parents is one of the most important steps in any breeding program. No selection method can extract good cultivars if the parents used in the program are not suitable. Therefore, emphasis was given to choose appropriate parents in order to obtain useful segregants. Breeders have different approach to choose parents and have achieved this in different ways. In common, inclusion of at least one locally adapted, popular cultivar as parent (White Ponni) will largely help to ensure the recovery of a high proportion of progenies with adaptation and quality that are acceptable to farmers. The selection of parents would be in such a way that each parent should have the ability to complement the weakness of other. Apart from this, the union of genetically dissimilar parents like Moroberekan would give better recombinants in the segregating progenies.

Selection of parents for the development of mapping population also depends on the performance of parents based on the earlier reports. White Ponni was a moderate yielder with 45 quintals / hectare; superior grain quality for cooking purpose and it is widely used in Tamil Nadu state, India and Southern Indian states. The variety suffers heavy yield losses due to leaf blast and panicle blast. Introgression of resistance genes for leaf blast

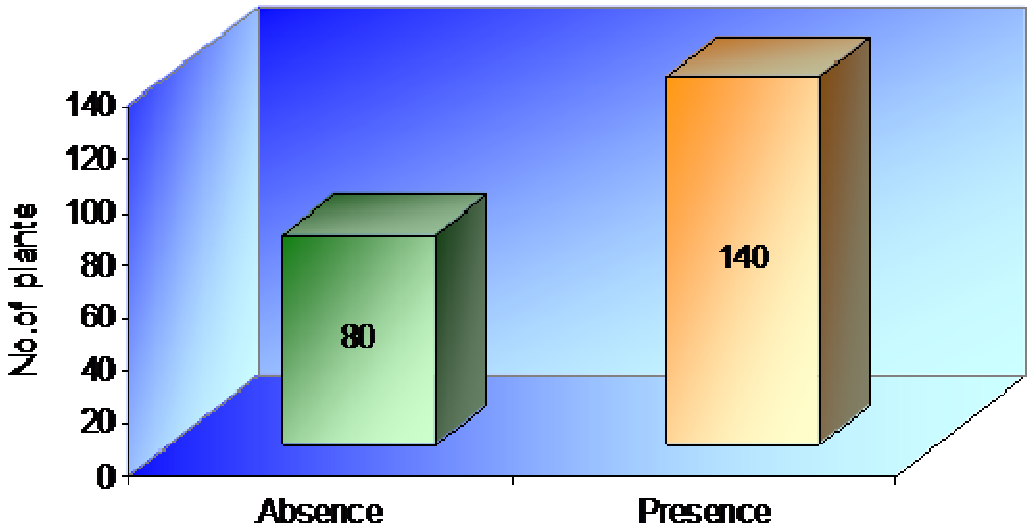

Figure 5. Frequency distribution of Glume pigmentation traits over the F_2 populations of Whte Ponni / Moroberekan.

Table 3. Variation for phenological traits among the F_2 mapping population of White Ponni / Moroberekan.

Morphological trait/ disease reaction	White Ponni (P_1)	Moroberekan (P_2)	Mean	Range	Sample Variance	Standard Error	Standard Deviation	Confidence (5 % / 1 %)
GPT/purple pigmentation	1**	3**	2.27**	1.00 - 3.00	0.93	0.065	0.96	0.128 / 0.169
Days to flowering	105**	91**	99.77**	71 - 126	159.05	0.85	12.61	1.670 / 2.210
Plant height (cm)	92.00**	87.00**	81.66**	48.00 – 125.00	224.31	1.01	14.98	1.990 / 2.620
Panicle length (cm)	18.60**	17.90**	20.59**	11.00 – 27.00	11.400	0.23	3.38	0.448 / 0.590
Grain yield/plant	53.80**	39.90**	45.81**	19.54 – 100.26	198.03	0.95	14.07	1.870 / 2.470
F3 population								
Leaf blast score (1 to 9)	8.36**	1.850**	4.24**	1.167 – 9.00	6.57	0.17	2.56	0.340 / 0.449
Potential disease incidence (PDI %)	92.88**	20.55**	47.12**	12.96 – 100.00	811.50	1.921	28.48	3.788 / 4.998

** - Confidence at 1 % level; GPT, Glume purple tip / purple pigmentation in culm region ('1' for absence; '3' if pigmentation is present).

might favour the variety to sustain yield losses.

Moroberekan is an upland japonica rice cultivar with apparently durable resistance that has been cultivated for many years in large areas of West Africa without losses to blast disease (Wang et al., 1994). Five resistance genes have been identified in African cultivar Moroberekan. Wang et al. (1994) located two major blast resistance loci, *Pi5(t)* and *Pi7(t)*, located on chromosomes 4 and 11, respectively, through restriction fragment length polymorphism (RFLP) analysis of the Moroberekan/CO39 recombinant inbred line (RIL) population. Using this same RIL population, Naqvi and Chattoo (1996) and Naqvi et al. (1995) identified and mapped two more major resistance loci, *Pi10(t)* and *Pi157*, on chromosomes 5 and 12, respectively. Chen et al. (1999) mapped the blast resistance gene *Pi-44(t)* using bulk segregant Amplified Fragment Length Polymorphism (AFLP) analysis.

Two dominant AFLP markers (AF_{348} and AF_{349}) linked to *Pi-44(t)* were identified. AF_{349} and AF_{348} were located at 3.3 ± 1.5 cM and 11 ± 3.5 cM from *Pi-44(t)*, respectively on chromosome 11.

The cultivar has been widely used as a resistance donor in many resistance breeding programs (Wang et al., 1994). It has been suggested that this stable resistance may be due to a combination of complete and partial resistance factors. The preliminary mapping effort by Wang et al. (1994) supported the view that a combination of complete and partial resistance factors may confer stable resistance, and provide a first step in allowing a more efficient utilization of these particular genes in future breeding efforts.

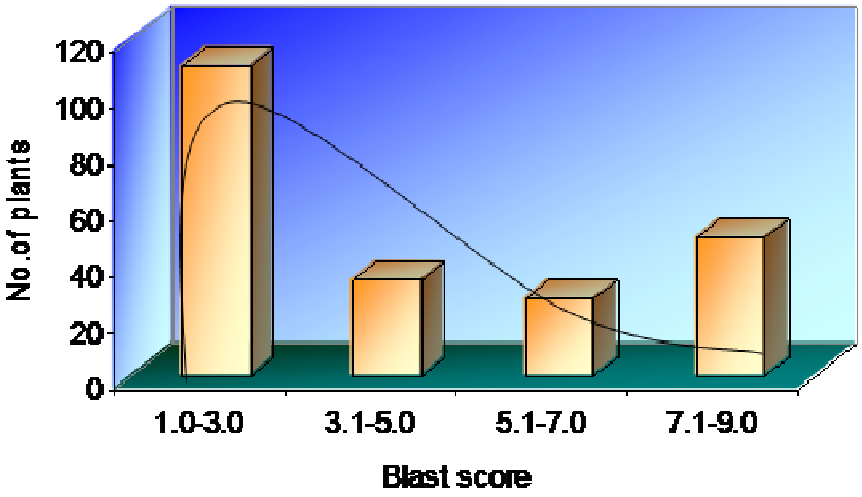

Figure 6. Frequency distribution of blast disease score over the 220 F_3 families of White Ponni x Moroberekan.

The results obtained based on the natural disease screening for partial leaf blast resistance indicated that Moroberekkan was a highly resistant genotype with a highly significant, low mean disease score and PDI%, respectively. Contrastingly, White Ponni was found to be a highly susceptible variety, as evidenced by the highly significant, higher mean disease reaction score and PDI % recorded.

Results obtained pertaining to the artificial screening for durable blast resistance also indicated that the genotype, Moroberekan recorded a highly significant lower mean disease score and mean PDI%. Contrastingly, White Ponni was found to be highly susceptible as evidenced by the highly significant, blast reaction scores and the PDI% obtained (Table 4).

Artificial screening showed variations for different scores of reaction, indicating the involvement of more than one resistance gene. Genetic analysis of blast resistance has indicated that many cultivars carry multiple genes for resistance. In this study, the F_3 population did not show a good fit to the expected genetic ratio (1:2:1) based on Chi-square test in the F_3 generation. Earlier studies indicated that either F_3 populations or backcross were used to confirm the expected genetic ratios (Kiyosawa, 1984; Yu et al., 1987). The number of resistant families (110) was higher than expected. The reasons for segregation distortion might be due to the presence of several resistance genes in a single cultivar, as the cultivar Moroberekan consisted of more than two resistance genes (Chen et al., 1999) which imparts more resistance. The increase in the number of resistance genes would decrease the proportion of plants susceptible to the isolates or the blast pathogen, thus making reliable detection of the segregation ratios more difficult (Yu et al., 1987).

The increased resistance or the increased level of dominance to leaf blast disease reaction observed in this study may be due to the presence of more than two resistance genes in the resistant parent, Moroberekan or might be due to the disease escape during screening.

Besides SSR markers, which usually produce single locus markers, RAPD markers could remain as an alternative marker system considering their ability to produce multi-locus markers. Seven RAPD primers viz., OPAL 16, OPAL 18, OPBA 15, OPBB 5, OPBB 13, OPBD 12 and OPM 9 were used to screen the 220 F_2 individuals of White Ponni / Moroberekan as they generated more number of markers which were clear and polymorphic than the other polymorphic RAPD primers.

The utility of RAPD analysis for genetic map construction was not fully exploited because of its inherent weakness in the technique. RAPD markers are preferred to Inter Simple Sequence Repeat (ISSR) and AFLP markers as their development costs and their running costs per data point were comparatively lower. The amount of DNA required is also lower when compared to other marker systems and conveniently agarose gel electrophoresis can be used instead of acrylamide, which might be carcinogenic if handled improperly.

Among the 12 RAPD markers scored, none of the markers deviated from the expected Mendelian segregation ratio of 3:1, except OPBB 5_{258} which skewed more towards Moreoberekan. The RAPD markers showed the overabundance of Moroberekan alleles. Since RAPD markers are of dominant in nature, they lack the power to discriminate the heterozygotic alleles from the dominant alleles in the F_2 mapping population. This was evident from the overabundance of resistant alleles (Moroberekan) rather than the recessive alleles (White Ponni).

The technical efficiency and multiplex potential of SSRs makes them preferable for many forms of high throughput mapping, genetic analysis by diversity studies and marker

Table 4. Leaf blast disease reactions recorded in the F_3 families of the cross White Ponni x Moroberekan under artificial condition

Plant No.	PDI%	Blast disease score	Disease reaction	Plant No.	PDI%	Blast disease score	Disease reaction	Plant No.	PDI%	Blast disease score	Disease reaction
1	27.04	2.43	R	42	91.67	8.25	S	83	99.99	9.00	S
2	16.30	1.47	R	43	48.89	4.40	MR	84	58.89	5.30	MS
3	20.56	1.85	R	44	14.81	1.33	R	85	27.78	2.50	R
4	19.63	1.77	R	45	81.30	7.32	S	86	24.81	2.23	R
5	54.26	4.88	MR	46	25.19	2.27	R	87	26.67	2.40	R
6	16.11	1.45	R	47	39.99	3.60	MR	88	27.78	2.50	R
7	26.48	2.38	R	48	17.78	1.60	R	89	57.41	5.17	MS
8	27.04	2.43	R	49	35.93	3.23	R	90	25.93	2.33	R
9	30.93	2.78	R	50	39.63	3.57	MR	91	45.56	4.10	MR
10	92.04	8.28	S	51	76.11	6.85	MS	92	92.22	8.30	S
11	30.74	2.77	R	52	42.96	3.87	MR	93	44.81	4.03	MR
12	91.11	8.20	S	53	89.63	8.07	S	94	23.52	2.12	R
13	91.48	8.23	S	54	40.00	3.60	MR	95	25.93	2.33	R
14	19.81	1.78	R	55	99.99	9.00	S	96	99.26	8.93	S
15	19.63	1.77	R	56	33.52	3.02	R	97	44.81	4.03	MR
16	97.04	8.73	S	57	77.04	6.93	MS	98	18.15	1.63	R
17	31.30	2.82	R	58	44.44	4.00	MR	99	27.78	2.50	R
18	21.85	1.97	R	59	26.11	2.35	R	100	25.93	2.33	R
19	32.59	2.93	R	60	20.00	1.80	R	101	86.67	7.80	S
20	38.33	3.45	R	61	16.30	1.47	R	102	75.37	6.78	MS
21	20.74	1.87	R	62	97.41	8.77	S	103	16.30	1.47	R
22	90.93	8.18	S	63	20.37	1.83	R	104	63.33	5.70	MS
23	68.70	6.18	MS	64	91.48	8.23	S	105	23.33	2.10	R
24	53.70	4.83	MR	65	95.93	8.63	S	106	25.93	2.33	R
25	23.15	2.08	R	66	17.41	1.57	R	107	79.26	7.13	S
26	33.52	2.72	R	67	81.48	7.33	S	108	96.67	8.70	S
27	36.67	3.80	R	68	25.19	2.27	R	109	17.41	1.57	R
28	21.85	1.97	R	69	99.99	9.00	S	110	31.30	2.82	R
29	15.93	1.43	R	70	91.48	8.23	S	111	17.41	1.57	R
30	15.93	1.43	R	71	73.70	6.63	MS	112	43.52	3.92	MR
31	21.85	1.97	R	72	24.81	2.23	R	113	65.19	5.87	MR
32	38.52	3.47	MR	73	42.22	3.80	MR	114	22.59	2.03	R
33	63.15	5.68	MS	74	28.15	2.53	R	115	18.89	1.70	R
34	18.52	1.67	R	75	18.52	1.67	R	116	43.33	3.90	MR
35	49.26	4.43	MR	76	26.11	2.35	R	117	77.41	6.97	MS
36	20.74	1.87	R	77	24.07	2.17	R	118	26.67	2.40	R
37	56.67	5.10	MR	78	30.74	2.77	R	119	33.51	3.02	R
38	55.19	4.97	MR	79	25.37	2.28	R	120	23.70	2.13	R
39	12.96	1.17	R	80	24.44	2.20	R	121	19.63	1.76	R
40	21.85	1.97	R	81	94.07	8.47	S	122	25.19	2.27	R
41	23.70	2.13	R	82	85.19	7.67	S	123	25.93	2.33	R

assisted plant improvement as they are co-dominant and multi-allelic (Coburn et al., 2002; Sharopova et al., 2002).

In the present investigation, among the two marker systems, SSR markers are preferred because of their abundance, technical simplicity, stable inheritance, high level of polymorphism and wide coverage of the whole genome (Chen et al., 1997). Nguyen et al. (2006) developed 190 F_2 progeny / F_3 families from a cross between Danghang-Shali (susceptible), an *indica* rice cultivar and Hokkai 188 (resistant), a *japonica* rice cultivar and they selected 126 F_2 progeny/F_3 families randomly for mapping

Table 4. Leaf blast disease reactions recorded in the F_3 families of the cross White Ponni x Moroberekan under artificial condition (contd...)

Plant No.	PDI%	Blast disease score	Disease reaction	Plant No.	PDI%	Blast disease score	Disease reaction	Plant No.	PDI%	Blast disease score	Disease reaction
124	93.70	8.43	S	167	99.99	9.00	S	210	23.33	2.10	R
125	26.30	2.37	R	168	70.37	6.13	MR	211	28.70	2.58	R
126	52.59	4.73	MR	169	99.99	9.00	S	212	37.96	3.42	MR
127	28.52	2.57	R	170	69.63	6.27	MS	213	35.00	3.15	R
128	23.70	2.13	R	171	30.19	2.72	R	214	91.11	8.20	S
129	95.19	8.57	S	172	48.89	4.40	MR	215	39.26	3.53	MR
130	92.96	8.37	S	173	95.93	8.63	S	216	22.59	2.03	R
131	24.44	2.20	R	174	19.63	1.77	R	217	72.77	6.55	MS
132	59.63	5.37	MS	175	21.48	1.93	R	218	28.52	2.57	R
133	92.59	8.33	S	176	25.93	2.33	R	219	32.22	2.90	R
134	54.07	4.87	MR	177	34.44	3.10	R	220	18.52	1.67	R
135	25.56	2.30	R	178	24.81	2.23	R				
136	48.15	4.33	R	179	27.78	2.50	R	P_1	98.88	8.9	S
137	57.78	5.20	MS	180	30.00	2.70	R	P_2	20.55	1.85	R
138	81.48	7.33	S	181	43.70	3.93	MR				
139	58.15	5.23	MS	182	31.10	2.80	R				
140	47.04	4.23	MR	183	21.48	1.93	R				
141	22.96	2.07	R	184	23.70	2.13	R				
142	37.22	3.35	R	185	92.22	8.30	S				
143	21.11	1.90	R	186	24.44	2.20	R				
144	99.99	9.00	S	187	19.63	1.77	R				
145	89.63	8.07	S	188	73.15	6.58	MS				
146	22.59	2.03	R	189	16.30	1.47	R				
147	24.07	2.17	R	190	26.30	2.37	R				
148	63.70	5.73	MS	191	92.59	8.33	S				
149	26.67	2.40	R	192	52.96	4.77	MR				
150	75.93	6.83	MS	193	70.00	6.30	MS				
151	53.89	4.85	MR	194	33.33	3.00	R				
152	19.63	1.77	R	195	93.33	8.40	S				
153	28.52	2.57	R	196	18.15	1.63	R				
154	16.67	1.50	R	197	99.99	9.00	S				
155	99.99	9.00	S	198	62.22	5.60	MS				
156	95.93	8.63	S	199	20.37	1.83	R				
157	33.15	2.98	R	200	76.85	6.92	MS				
158	90.00	8.10	S	201	57.41	5.17	MS				
159	17.78	1.60	R	202	22.22	2.00	R				
160	96.67	8.70	S	203	94.07	8.47	S				
161	17.04	1.53	R	204	99.99	9.00	S				
162	64.81	5.83	MS	205	26.30	2.37	R				
163	19.63	1.77	R	206	99.99	9.00	S				
164	19.26	1.73	R	207	27.78	2.50	R				
165	20.74	1.87	R	208	93.33	8.40	S				
166	66.48	5.98	MS	209	17.78	1.60	R				

R- Resistant (1.00 – 3.00),
MR- Moderately resistant (3.1 to 5.0),
MS – Moderately susceptible (5.1-7.0)
S – Susceptible (7.1-9.0), P1 – Female parent (White Ponni);
P_2; Male parent (Moroberekan).

survey.

Linkage map construction of any organism depends on the availability of the segregating markers covering the entire genome. In the present study, 57 SSR primer pairs which gave clear un-ambiguous polymorphic banding during the parental survey were used on 220 F_2 individuals. Of the 57 primer pairs, only 52 primer pairs could be scored, with clear scorable banding pattern. The reduction occurred since the 5 SSR primers surveyed on the F_2 population could not be scored due to their complex banding pattern.

Out of 52 marker loci, 44 (84.61%) fitted into the expected segregation ratio of 1:2:1 based on χ^2 - test at 0.05% probability value. Out of 8 markers which deviated from the Mendelian segregation ratio, 5 exhibited segregation distortion towards Moroberekan and 3 skewed towards White Ponni. 61.53% of the individuals had more heterozygotic alleles for 52 markers surveyed. The overall allele frequency for the SSR loci showed an overabundance of heterozygotic alleles and a low level segregation distortion of genetic markers was observed, since the cross combination of distantly related parents were involved.

Wang et al. (1994) observed segregation distortion for almost 127 RFLP markers used in the recombinant inbred population of CO 39, an *indica* variety and Moroberekan, a *japonica* variety. Sirithunya et al. (2002) reported that out of 121 marker loci, 93 showed significant segregation distortion ($p \leq 0.05$) in a study conducted to find out the QTLs associated with leaf and neck blast resistance in RIL population of rice. The distortion of markers could be either due to lethal alleles in gametes or competition among gametes and abortion of gamete or zygote or due to the sampling error (Pillen et al., 1992).

In the present investigation, the percentage of segregation distortion (15.38%) was comparatively lower for SSR markers, when compared to the earlier reports (Wang et al., 1994; Sirithuniya et al., 2002). This might be due to the parents involved in the generation of mapping population, as the susceptible parent, White Ponni (Taichung 65/2 x Mayang Ebos - 80) consisted of Taichung 65/2 which is a *japonica* type and the other parent Moroberekan is also a *japonica* type. Hence the percentage of segregation distortion might be lower as expected since the parents involved were of the same type (*japonica* type).

Although, it is not possible to construct a genetic linkage map covering the entire genome of rice with the available 52 SSR markers, an attempt was made to develop different linkage groups by performing a linkage analysis. Linkage groups were established for all the chromosomes except chromosome 9. Interestingly, no leaf blast resistance gene was reported so far in chromosome 9 (Sallaud et al., 2003). The multipoint analysis carried out on the marker data of the 52 segregating SSRmarkers resulted in the linkage map with 40 loci.

When two point analyses were done involving 52 SSR markers and 12 RAPD markers, it was observed that all of the RAPD markers were either linked with SSR markers or with the RAPD markers. Out of 12 RAPD markers, only 3 R (OPBD 12_{600}, OPBB 5_{258} AND OPBB 5_{194}) were found to be linked with SSR markers and the remaining markers paired themselves. OPBD 12_{600} and OPBB 5_{194} was linked with RM1309 on chromosome 8, but these two RAPD markers paired between them making the situation complex to place the RAPD markers on the specific chromosome. The cause for not having the accurate linkage map could be due to the skewness in the population for marker allele distribution. Smaller number of recombinants in a population due to random assortment or non-linkage might be the major limiting factors to accurately assemble linkage groups (Wang et al., 1994).

In this study, an attempt was made to establish the genotype-phenotype association involving SSR and RAPD markers and six plant traits. Though it was possible to have a genetic linkage map of White Ponni/ Moroberekan with 52 SSR markers in the present investigation, the map was very sparse and incomplete. Considering the situation, the QTL analysis for all the six phenotypic characters was carried out using the SMA. The SMA is a good start not only for learning QTL mapping, but also for practical data analysis. Single marker analysis is the method used in earliest studies on QTL mapping (Edwards et al., 1987; Weller et al., 1988). In this, one marker is considered at a time to find the QTL marker association. The SMA can be implemented as a simple ANOVA, linear regression, t-test and maximum likelihood estimation (Haley and Knott, 1992). SMA is simple in terms of data analysis and implementation. It can be performed using common statistical software like Microsoft EXCEL. Marker orders and complete linkage map is not required.

Based on the phenotypic values of the parents, it is evident that Moroberekan is more resistant to leaf blast than White Ponni. White Ponni is more efficient in the single plant yield than Moroberekan along with the other associated traits. Wider variations with transgressive segregation for different traits were observed among the F_2 families indicating the quantitative nature of the traits.

The results indicated that number of SSR markers associated with phenotypic traits ranged from 2 (plant height) to 9 (purple pigmentation). Some of the SSR markers were linked for more than one trait in this study. The marker phenotype commonality was found on chromosomes 3, 4, 5 and 6. The detection of common markers for the earlier mentioned parameters suggested that correlated traits might be influenced by common chromosomal regions.

To identify the common QTLs across genetic backgrounds, the results of the present study are compared with similar QTL studies on other populations. In the present study, QTL for leaf blast resistance was detected on chromosome 2 and 4. Similar results were obtained by

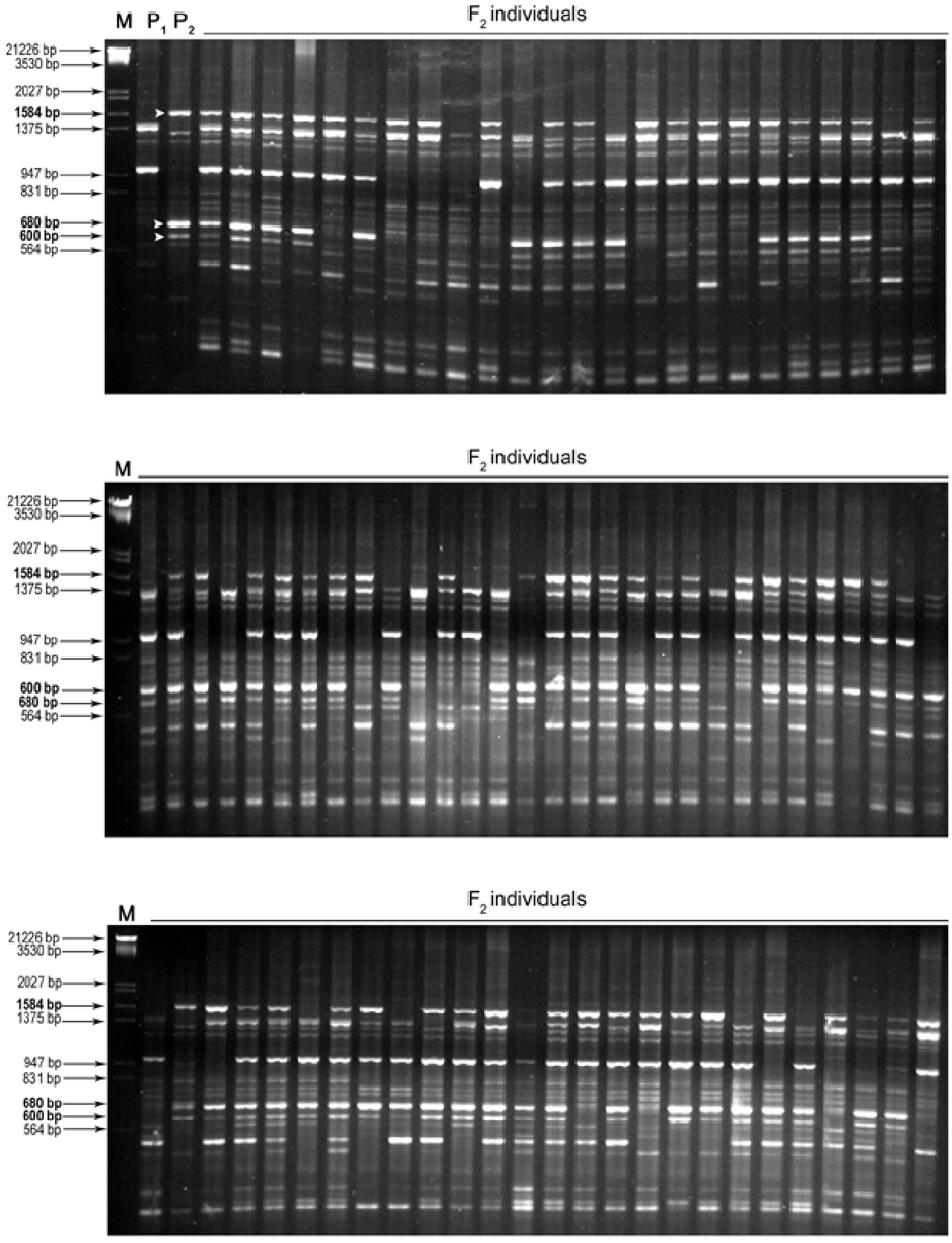

Figure 7. OPBD $12_{600,\ 680}$ and OPBD 12_{1584} marker segregation among the individual F_2 lines of White Ponni (P_1) / Moroberekan (P_2); M - Marker; Lambda DNA / *Eco*RI+*Hin*dIII, P_1 - White Ponni, P_2 – Moroberekan.

Table 5. Segregation pattern of RAPD markers in F_2 mapping population.

S/N	Marker	Observed value		$(O - E)^2 / E$ (1)	$(O - E)^2 / E$ (2)	χ^2 value
		Score 0	Score 1			
1	OPAL16 (940)	59	161	0.291	0.097	0.388**
2	OPAL18 (1054)	64	156	1.473	0.491	1.964**
3	OPBA 15 (458)	67	153	2.618	0.873	3.491**
4	OPBA 15 (742)	51	169	0.291	0.097	0.388*
5	OPBD 12 (680)	58	162	0.164	0.055	0.219**
6	OPBD 12 (600)	56	164	0.018	0.006	0.024**
7	OPBD 12 (1584)	54	166	0.018	0.006	0.024**
8	OPM9 (350)	63	157	1.164	0.388	1.552**
9	OPBB 5 (194)	62	158	0.891	0.297	1.188**
10	OPBB 5#(258)	38	182	5.255	1.752	7.007
11	OPBB 5 (700)	60	160	0.455	0.152	0.607**
12	OPBB 13 (755)	71	149	4.655	1.552	6.207**

$\chi 2$ value: 3.84 at P ≤ 0.05 and 6.63 at P ≤ 0.01; #, deviation of the marker from Mendelian segregation ratio 3:1.

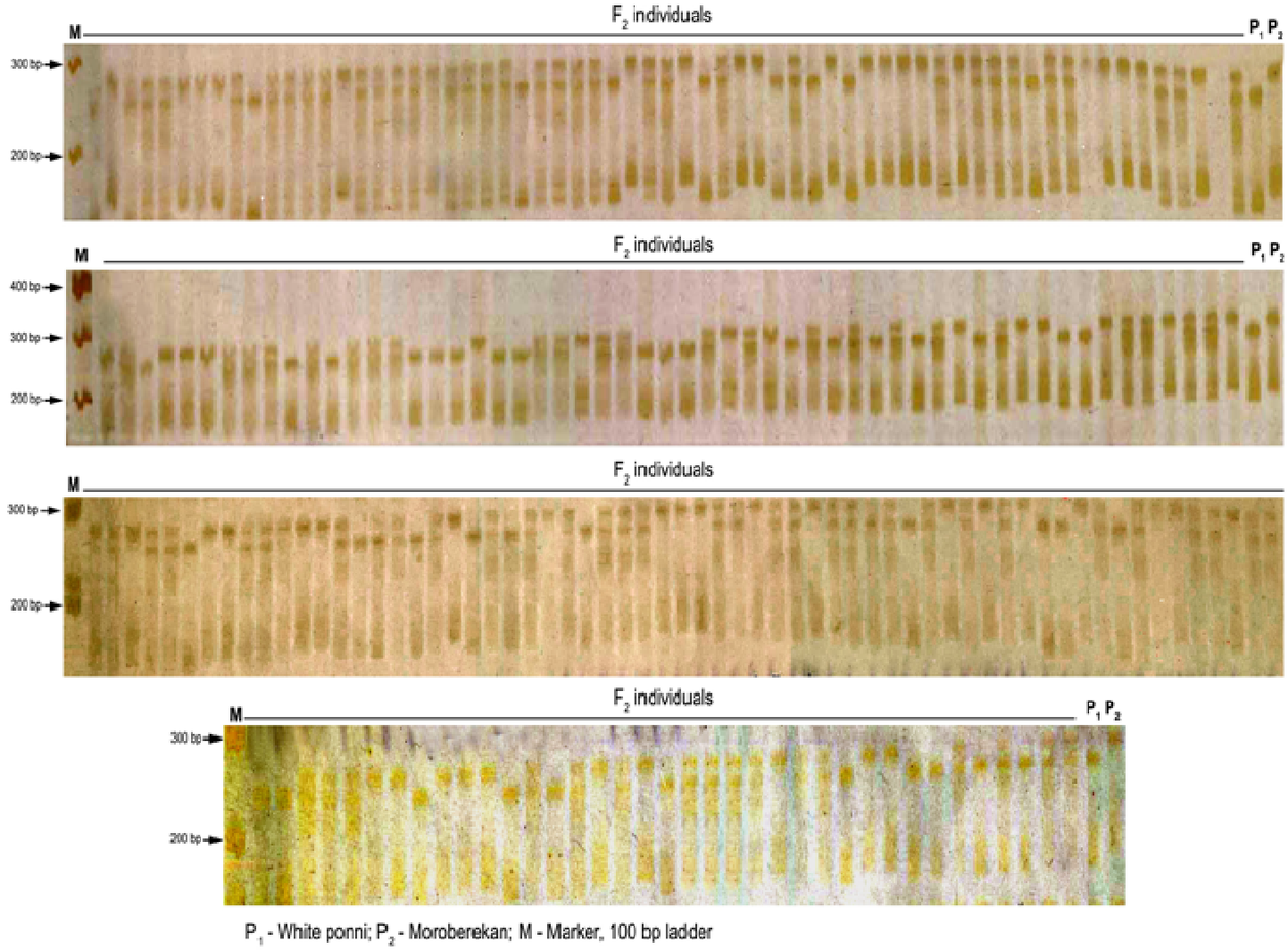

Figure 8. Segregation pattern of SSR marker RM 451 in the F_2 individuals of White Ponni / Moroberekan.

Table 6. Segregation pattern of SSR markers in F_2 mapping population.

S/N	Marker	Observed value			$(O - E)^2 / E$			χ^2 value
		Score 1	Score 2	Score 3	(1)	(2)	(3)	
1	RM 81a#	48	0	172	0.891	110.000	248.89	359.78
2	RM 84#	47	0	173	1.164	110.000	253.16	364.32
3	RM 472	47	122	51	1.164	1.309	0.291	2.764**
4	RM 579	42	117	61	3.073	0.445	0.655	4.173**
5	RM 582	38	122	54	5.255	1.309	0.018	6.582*
6	RM 6466#	33	126	61	8.800	2.327	0.655	11.782
7	RM 5638#	33	119	68	8.800	0.736	3.073	12.609
8	RM 8053	40	119	61	4.091	0.736	0.655	5.482**
9	RM 8070	41	118	61	3.564	0.582	0.655	4.801**
10	RM 211	60	100	60	0.455	0.909	0.455	1.819**
11	RM 279	54	103	63	0.018	0.445	1.164	1.627**
12	RM 492	45	117	58	1.818	0.445	0.164	2.427**
13	RM 168	39	114	67	4.655	0.145	2.618	7.418*
14	RM 411	43	116	61	2.618	0.327	0.655	3.600**
15	RM 545	54	100	66	0.018	0.909	2.200	3.127**
16	RM 1002	52	100	68	0.164	0.909	3.073	4.146**
17	RM 3202	68	99	53	3.073	1.100	0.073	4.246**
18	RM 280	56	115	49	0.018	0.227	0.655	0.900**
19	RM 451	59	103	58	0.291	0.445	0.164	0.900**
20	RM 551	47	113	60	1.164	0.082	0.455	1.701**
21	RM 5757	53	110	57	0.073	0.000	0.073	0.146**
22	RM 122	51	118	51	0.291	0.582	0.291	1.164**
23	RM 574	42	128	50	3.073	2.945	0.455	6.473*
24	RM 1237	45	132	43	1.818	4.400	2.618	8.836*
25	RM 402	39	123	58	4.655	1.536	0.164	6.355*
26	RM 584	42	121	57	3.073	1.100	0.073	4.246**
27	RM 585	40	123	57	4.091	1.536	0.073	5.700**
28	RM 1163	46	129	45	1.473	3.282	1.818	6.573**
29	RM 70	50	111	59	0.455	0.009	0.291	0.755**
30	RM 432	60	117	43	0.455	0.445	2.618	3.518**
31	RM 1048#	47	0	173	1.164	110.000	253.164	364.328
32	RM 1279	52	115	53	0.164	0.227	0.073	0.464**
33	RM 2878#	169	0	51	236.291	110.000	0.291	346.582
34	RM 3186	51	119	50	0.291	0.736	0.455	1.482**
35	RM 3555	55	120	45	0.000	0.909	1.818	2.727**
36	RM 3583	52	119	49	0.164	0.736	0.655	1.555**
37	RM 4584	47	118	55	1.164	0.582	0.000	1.746**
38	RM 5508	48	123	49	0.891	1.536	0.655	3.082**
39	RM 80	63	104	46	1.164	0.327	1.473	2.964**
40	RM 1309	55	126	39	0.000	2.327	4.655	6.982**
41	RM 4955	60	109	51	0.455	0.009	0.291	0.755**
42	RM 7285#	57	131	32	0.073	4.009	9.618	13.700
43	RM 566#	144	29	47	144.018	59.645	1.164	204.827
44	RM 5102	52	119	49	0.164	0.736	0.655	1.555**
45	RM 216	54	108	58	0.018	0.036	0.164	0.218**
46	RM 244	73	95	52	5.891	2.045	0.164	8.100*
47	RM 206	59	114	47	0.291	0.145	1.164	1.600**
48	RM 209	66	111	43	2.200	0.009	2.618	4.827**
49	RM 332	50	107	63	0.455	0.082	1.164	1.701**
50	RM 3331	49	113	58	0.655	0.082	0.164	0.901**
51	RM 4589	53	109	58	0.073	0.009	0.164	0.246**
52	RM 5341	63	97	60	1.164	1.536	0.455	3.155**

χ^2 value: 5.99 at $P \leq 0.05$ and 9.21 at $P \leq 0.01$; O, observed value; E, expected value; #, primers deviating from Mendelian segregation ratio (1: 2:1).

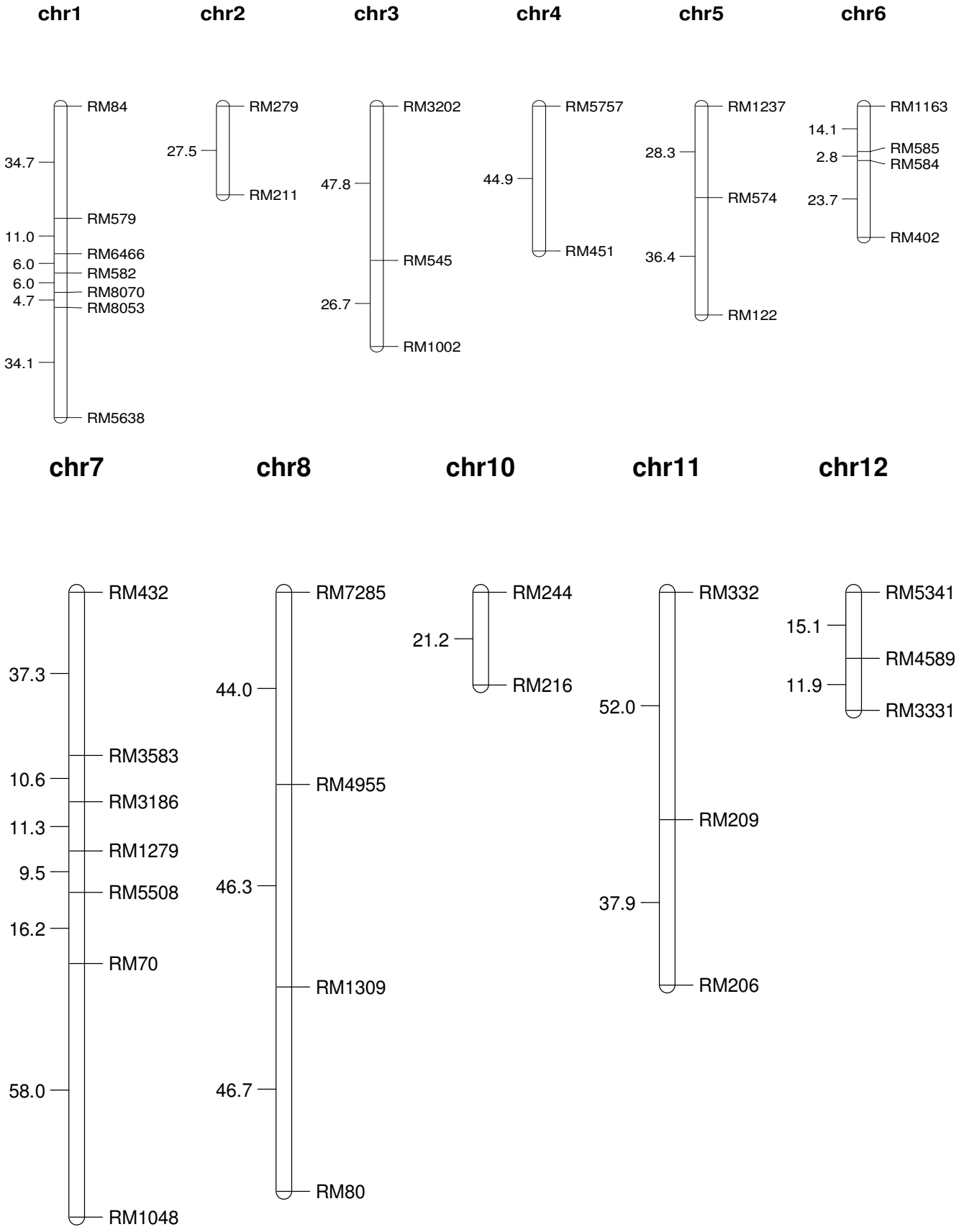

Figure 9. Frame work linkage map of White Ponni/ Moroberekkan F_2 population for SSR markers surveyed.

Table 7. Summary of SSR and RAPD marker loci putatively associated with phenotypic traits identified by single marker analysis and simple regression analysis.

Markers	Chromosome	F (Cal)	Pr > F	R^2 (%)
Markers linked to leaf blast resistance				
RM 5757	4	219.64**	< 0.0001	66.9
RM 451	4	16.09**	< 0.0001	12.9
OPBB 5 (258 bp)	-	14.993**	0.0001	6.40
OPBB 5 (194 bp)	-	8.764**	0.003	3.90
RM 492	2	6.375**	0.002	5.50
OPAL 16 (940 bp)	-	5.175**	0.024	2.32
OPBD 12 (680bp)	-	4.620*	0.033	2.10
Markers linked to Glume Purple Tip (GPT) and purple pigmentation				
RM 402	6	26.072**	< 0.0001	19.40
RM 585	6	24.452**	< 0.0001	18.40
RM 584	6	24.191**	< 0.0001	18.20
RM 1163	6	12.078**	< 0.0001	10.00
OPAL 16 (940 bp)	-	7.327**	0.007	3.30
OPBD 12 (680 bp)	-	6.767**	0.010	3.00
OPAL 18 (1054 bp)	-	6.655**	0.011	3.00
RM 3202	3	6.467**	0.002	5.60
RM 6466	1	4.901**	0.008	4.30
RM 582	1	4.854**	0.009	4.30
RM 8053	1	4.511*	0.008	4.30
OPBD 12 (600 bp)	-	4.235*	0.041	1.90
OPBB 13 (755 bp)	-	4.220*	0.041	1.90
RM 8070	1	3.789*	0.024	3.40
Markers linked to days to flowering				
RM 585#	6	7.982**	0.0004	6.85
RM 584#	6	6.78**	0.001	5.88
RM 1163#	6	5.033**	0.007	4.43
RM 3202#	3	4.707*	0.001	4.10
RM 574#	5	3.997*	0.020	3.60
RM 402#	6	3.282*	0.039	2.90
Markers linked to plant height				
RM 574#	5	4.893**	0.008	4.31
RM 1237	5	4.093*	0.0178	3.60
Markers linked to panicle length				
RM 451#	4	42.194**	< 0.0001	28.00
RM 2878	7	6.515**	0.011	2.80
RM 566	9	5.835**	0.003	5.10
RM 4584	7	3.105*	0.046	2.78
Markers linked to grain yield per plant				
RM 1279	7	20.604**	< 0.0001	16.00
RM 3186	7	11.771**	< 0.0001	10.00
RM 70	7	10.097**	< 0.0001	8.50
RM 5508	7	10.039**	< 0.0001	8.50
OPAL 16 (940 bp)	-	7.909**	0.005	3.50
RM 3583	7	3.815*	0.024	3.40
RM 80	8	3.414*	0.035	3.15
RM 574	5	3.228*	0.042	2.90

F-critical value: 5% level, 3.037*; 1% level, 4.704**. #, markers linked for more than one trait.

Sallaud et al. (2003) who located resistance loci (RL) on chromosome 1, 2, 5, 6, 7, 10, 11 and 12. Wu et al. (2005) identified 10, 6 and 7 QTLs for percent diseased leaf area (percent DLA), lesion number (LN) and lesion size (LS) located on chromosomes 1, 2, 3, 7, 8 and 11 for percent DLA, 1, 2, 3, 8 and 11 for LN, and 1, 2, 3, 8, 10 and 11 for LS, respectively.

Major resistance genes for leaf blast resistance were located on chromosomes 2 and 4 as reported earlier by various workers. Wang et al. (1994) reported *Pi-5(t)*; amajor resistance gene was located in chromosome 4 flanked by the markers RG788 and RG864. Fukuoka and Okuno (2001) reported that the resistance gene *Pi-21* was located on the chromosome 4 flanked by the markers G271 and G317. Later, Jeon et al. (2003) reported that the same resistance gene *Pi-5(t)* was located in chromosome 9 and not in chromosome 4. Sallaud et al. (2003) reported that the marker RG520 was located closer to the resistance gene *Pi-25(t)* located onchromosome 2. The resistant donor used in both studies was Moroberekan.

Linked markers for glume purple tip (GPT) or the pigmentation character was detected on the chromosomes 1, 3 and 6 by single marker analysis. The QTL for days to 50$% flowering was located on chromosomes 3, 5 and 6. Similar results were obtained for days to flowering by Yano and Sasaki (1997) on chromosome 5, Xiao et al. (1995) on chromosome 3, 4, 7 and 8, Yamamoto et al. (1998) on chromosome 6 and 7, Brondani et al. (2002) on chromosome 2, 3 and 7, and Xiao et al. (1998) on chromosome 1, 3, 5, 6, 7, 8 and 12. Linked markers for plant height was detected on chromo-some 5, similar results were reported by Xiao et al. (1995) and Babu et al. (2003) on chromosome 5.

In the present investigation, panicle length was located on chromosomes 4, 7 and 9. Similar results were indicated by Xiao et al. (1995) on chromosomes 4 and 8, Xiao et al. (1998) on chromosomes 1, 2, 4, 8, 9 and 12 and Brondani et al. (2002) on chromosomes 4, 11 and 8.

Practically, the formation of yield is a very complex physiological pathway and genetic dissection of yield seems considerably difficult (Guo et al., 2005). Complex yield character may be regarded as the end-point of a process of which the successive stages are represented by observed yield attributing traits like productive tillers per plant, grains per panicle, percentage seed set and test weight (Piepho, 1995). In the present study, markers linked for yield was detected on chromosomes 5, 7 and 8. Similar results were obtained by Xiao et al. (1998) reported on chromosomes 2, 4, 5, 8 and 12, Brondani et al. (2002) on chromosomes 1, 2, 3, 4, 7 and 11 and Guo et al. (2005) who reported that the QTLs governing the grain yield per plant were located on chromosome 1, 2, 3, 5, 7 and 10.

At present, the phenotypic screening for blast has its own limitation as the exchange of materials between countries would generate more important information on the genetics of blast resistance and race distribution. Since the pathogen cannot be imported easily from one country into the other participating countries, the exchange is restricted only to seeds, since strict quarantine measures are employed. Nevertheless, numerous varieties have been exchanged between different countries for various experimental purposes, but the gene analysis of the host has not attracted the blast workers. To determine the relationship between genes effective in each country, seeds of F_3 lines or advanced generations of the hybrid between extremely resistant and susceptible varieties might be suitable (Kiyosawa, 1981).

Conclusion

Although different responses have been shown to induce resistance to different groups of pathogens, there are overlapping responses that confer protection against a wide range of diseases (Paul et al., 2000). Thus, it is clear that plant defense is very complex and involves multiple signaling pathways. Nevertheless, better understandings of these complex pathways are now possible by the development of new approaches and genetic tools such as mutants and microarray technology.

Abbreviations

Marker assisted selection; **RAPD,** random amplified polymorphic DNA; **SSR,** simple sequence repeats; **SMA,** single marker analysis; **QTL,** quantitative trait loci; **GPT,** glume pigmentation trait; **MR,** moderately resistant; **MS,** moderately susceptible; **RFLP,** restriction fragment length polymorphism; **RIL,** recombinant inbred line; **AFLP,** amplified fragment length polymorphism; **ISSR,** inter simple sequence repeat; **RL,** resistance loci; **DLA,** diseased leaf area; **PDI %,** potential disease incidence.

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