Electronic Journal of Plant Breeding, 2(1):8-23 (Mar 2011)
ISSN 0975-928X

Research Article

Marker Assisted Selection for Leaf Blast Disease resistance and other yield traits in rice (*Oryza sativa* L.) using RAPD Markers

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(Received:01 Nov 2010; Accepted:06 Dec 2010)

Abstract:
A segregating population of 220 F_2 individuals was developed by raising the selfed F_1 seeds of a single hybrid plant, White Ponni x Moroberekan. Two hundred and twenty F_3 families were phenotyped for blast resistance under artificial condition. Bulk-segregant analysis resulted in the identification of 12 leaf blast resistant phenotype specific RAPD markers. Mapping survey was done with seven RAPD primers which co-segregated in BSA. Twelve RAPD markers were scored in the F_2 mapping population. Out of 12 marker loci, 11 marker loci (91.97 per cent) fitted into the expected segregation ratio of 3:1 based on χ^2 - test at 0.05 per cent probability value. One way ANOVA was performed to identify the marker phenotype association and this resulted in the identification of seven RAPD markers linked to three traits *viz.,* leaf blast resistance (OPBB 5_{258}, OPBB 5_{194}, OPAL 16_{940} and OPBD 12_{680}), five markers for GPT and one marker for grain yield per plant.

Key Words: Bulked-segregant Analysis, Single-marker Analysis, *Magnaporthe grisea* (Hebert) Barr; Moroberekan; Grain yield, Glume Purple Tip.

Introduction

Rice (*Oryza sativa* L.), one of the world's most important cereal crop, is the primary calorie source for one-third of the world's population (Shim *et al*. 2004). More than 90% of the world's rice is grown and consumed in developing countries (Sirithunya *et al*. 2002). Rice blast, caused by the filamentous ascomycete *Magnaporthe grisea* (Hebert) Barr, is one of the most devastating diseases of rice (Zeigler *et al.*, 1994). The rice (*Oryza sativa* L.) – *Magnaporthe grisea* (Hebert) Barr patho-system is a nice model system to study plant fungus interactions (Berruyer *et al*. 2003). The disease causes heavy yield losses ranging from 35 to 50% during the epidemic years (Padmavathi *et al*. 2005). Although chemical control of the disease is possible, yet it remains economically unviable for resource-poor farmers and is environmentally unsafe. Cultivation of resistant varieties is an effective approach to eliminate the use of pesticides and minimize crop losses due to this disease. So far, about 50 major blast resistance genes have been identified and utilized in developing resistant cultivars (Liu *et al*. 2005).

However, blast resistance conferred by resistance genes is often short lived due to the emergence of virulent races of the pathogen which negate the effect of introduced resistance genes (Zeigler *et al*. 1995). Nonetheless, the major gene resistance to blast has been useful and should continue to be important in rice production if resistance genes are carefully selected and managed (Chen *et al*. 1996). In contrast, breeding for cultivars that display broad-spectrum resistance has become a priority for crop improvement. The genetic basis of broad-spectrum resistance is still not well understood. It may be controlled by single genes or multiple genes with cumulative effects (Johnson, 1981). Strategies that would create more durably resistant cultivars are presently focusing on the pyramiding of multiple resistance genes into susceptible cultivars (Hittalmani *et al*. 2000). Rice blast disease follows a classical gene-for-gene system (Silué *et al*. 1992; Valent, 1997), in which a pathogen strain expressing an avirulence (*AVR*) gene triggers the corresponding resistance (*R*) gene-mediated defense response.

Incorporation of individual *R* genes into existing rice cultivars has not achieved reliable, long lasting resistance to blast disease because of the high potential for *AVR* gene variation in the pathogen (Valent, 1997).

With the advent of recombinant DNA technologies, many DNA-based molecular markers have been developed and used extensively in marker assisted breeding, gene tagging, constructing genetic and physical linkage maps and marker-assisted selection in rice (McCouch *et al*. 1997). These molecular maps provide a base for the identification of blast resistance genes without the need for phenotypic selection that is normally used in classical breeding. Molecular marker analysis is useful for identification of genomic segments contributing to the genetic variance of a trait, and selection of superior genotypes. Marker analysis results in accurate genotypic information, which would be unavailable otherwise, giving a precision lacking with phenotypic measurements due to environmental interaction and experimental error (Altinkut *et al*. 2003).

Wang *et al*. (1994) made the first attempt to identify and locate QTL for blast resistance in Moroberekan, a durable resistant cultivar, as one of the resistance genes *Pi7(t)* was identified through screening with *M. grisea* and RFLP mapping of the recombinant inbred line population derived from a cross between the rice cultivars Moroberekan and CO39. *Pi7(t)* was positioned on the long arm of chromosome 11 near the bacterial blight resistance locus *Xa21*. Linkage analysis for blast resistance genes suitable for use in tropical regions has also been reported (Naqvi *et al*. 1995). Two resistance genes *Pi-b* (Wang *et al*. 1999) and *Pi-ta* (Bryan *et al*. 2000) have been isolated from the rice genome. However, very limited information is available on the molecular mapping of resistance genes against Indian blast races. Sallaud *et al* (2003) inoculated a rice progeny derived from the cross IR64 × Azucena with different *Magnaporthe grisea* isolates that showed differential responses on the parental cultivars. By QTL mapping, nine unlinked loci conferring resistance to each isolate were identified and named *Pi-24*(*t*) to *Pi-32*(*t*). They could correspond to nine specific resistance genes. The segregation analysis of an F_2 progeny of a cross between a susceptible cv. 'HPU741' and the resistant genotype 'DHR9' suggested that the resistance was governed by a single dominant gene. A RAPD marker, $OPA8_{2000}$, linked to the resistance gene was identified by the linkage analysis of 109 F_2 individuals (Kumar *et al*. 2010)

Blast resistance in rice cultivars are generally classified into two types, qualitative (complete) and quantitative (partial) (Fukuoka and Okuno 2001; Zenbayashi et al. 2002). Most of the partial resistances are non-race specific, quantitative and polygenic (Fukuoka and Okuno 2001; Zenbayashi *et al*. 2002). However, there are some exceptions such as Pb1 in cultivar Asanohikari (Fujii *et al*. 2000) and Pi21 in cv. Owarihatamochi (Fukuoka and Okuno 2001), which are single genes conditioning partial resistance to rice blast. Relatively broad spectrum or durable resistance has been observed in some rice cultivars. For example, the traditional African cultivar Moroberekan has been cultivated for many years in large areas of West Africa without high losses from blast (Bonman and Mackill, 1988). ROK16, LAC23, IRAT13, OS6 and some Brazilian upland rice cultivars show durable resistance to blast in upland conditions (Bonman and Mackill 1988; Ahn 1994; Fomba and Taylor 1994). Tetep, an indica rice cultivar, and Pai-Kan-Tao (PKT), a temperate japonica cultivar, both exhibit broad-spectrum resistance to rice blast (Mackill and Bonman 1992; Ahn 1994, 2000). Many of these rice lines have been used as resistance donors in breeding programs (Mackill and Bonman1992; Inukai *et al*. 1994).

RAPD markers developed by Williams *et al*. (1990), relies on the differential enzymic amplification of small DNA fragments using PCR with arbitrary oligonucleotide primers (usually 10-mers). Polymorphisms result from either chromosomal changes in the amplified regions or base changes that alter primer binding. The procedure is rapid, requires only small amounts of DNA, which need not be of high quality, and involves no radioactivity. Polymorphisms can be detected in fragments containing highly repeated sequences; this provides markers in regions of the genome previously inaccessible to analysis. The RAPD markers are usually dominant because polymorphisms are detected as the presence or absence of bands. RAPD markers provide a quick method for generating genetic maps and analyzing populations (Salem e*t al*. 2007).

In bulked segregant analysis, a population is screened for a character of interest and the genotypes at the two extreme ends form two bulks. Then the two bulks were tested for the presence or absence of molecular markers. Since the bulks are supposed to contrast for alleles contributing positive and negative effects, any marker polymorphism between the two bulks indicates the linkage between the marker and character of interest. Plants from segregating populations can also be grouped according to

phenotypic expression of a trait and tested for differences in allele frequency between the population bulks (Quarrie *et al*., 1999).
The strength of bulked segregant analysis (BSA) is that it does not require a linkage map and can be used where insufficient polymorphism exists for a map. An additional advantage is that the approach relies on the dramatic reduction in the number of marker assays when compared to building a genetic map for the purpose of identifying markers associated with a phenotype (Wenzl *et al*. 2007). Bulked segregant analysis has been employed by different scientists for identifying linked markers for disease resistance and other associated traits recently (Chen *et al*. 1999; Govindaraj *et al*. 2005; Selvaraj *et al*. 2009; Kanagaraj *et al*., 2010; Parihar *et al*. 2010; Araújo *et al*. 2010).

The objectives of the present investigation were to identify the RAPD markers linked to the leaf blast resistance and other yield contributing traits of rice in a $F_{2:3}$ population of a cross between a West African land race Moroberekan and a well known cultivar White Ponni of Southern Tamil Nadu, India.

Material and Methods:
Hybridization was effected at paddy breeding station, Coimbatore, Tamilnadu, in the year 2005, with the leaf blast resistant cultivar, Moroberekan (A West African cultivar), as a pollen parent and the susceptible cultivar, White Ponni (selection from the cross derivative, Taichung 65/2 x Mayang Ebos - 80) as an ovule parent. 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 (SSR and RAPD). A total of 220 selfed seeds of a single F_1 plant were collected and raised as F_2 to generate segregating population. Leaves were collected from all the 220 F_2 individuals for DNA extraction. All the 220 F_2 plants were selfed to collect the F_3 seeds. In the segregating population (White Ponni / Moroberekan) the following biometric observations *viz.,* days to 50 per cent flowering, plant height, presence or absence of pigmentation at the tip of the spikelets / Glume (GPT) / culm region, 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 at Paddy Breeding Station, Coimbatore 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 4–5 times at intervals. The screen house was maintained at 32 - 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. 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 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 1-2 millimeters in diameter with a distinct brown margin; score 5 - Narrow or slight elliptical lesions, 1-2 mm in breadth, more than 3mm 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 (PDI %) per cent was worked out using the formula by McKinney (1923): Sum of numerical rating / Number of leaves observed X 100 / Maximum disease grade.

Isolation of DNA, RAPD analysis and generating Bulked Segregant population
Fresh leaf samples collected from 15 days old seedlings of parental genotypes and the segregating population were used for isolation and purification of total genomic DNA following the method of McCouch *et al*. (1988). 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 mins for one cycle, 94 °C 1 min, 37 °C 1 min and 72 °C 2 mins for 40 cycles, then 72 °C 5 mins. 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).

A total of 266 RAPD primers of 16 series *viz.,* OPA, OPC, OPE, OPI, OPK, OPL, OPM, OPN, OPP,

OPAG, OPAL, OPBA, OPBB, OPBC, OPBD and OPBE were used for parental genotyping in this study. 200 RAPD primers which showed polymorphism between White Ponni and Moroberekan were used in Bulk Segregant Analysis (BSA). Then, PCR analysis was carried out by the bulked-segregant analysis (BSA) method by pooling 10 resistant and 10 susceptible F_2 individuals. The individuals were selected based on phenotypic scores obtained from the corresponding highly resistant (score < 3) and highly susceptible (score 9) F_3 progenies for leaf blast resistance (Jia *et al*. 2000). Individuals used for making bulk were screened with RAPD primers which gave phenotypic specific bands in both parents and respective bulks using the same temperature profile and same PCR ingredients.

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 two classes defined by RAPD markers for different quantitative traits to establish phenotype – marker association as suggested by Beckmann and Soller (1996) and Edwards *et al.* (1987) using the general linear model (GLM) procedure of the 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 QTL at or the marker locus. Simple regression analysis was also performed according to the method described by Haley and Knott (1992) using regression coefficient as 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 0 and 1 for RAPD) as independent variables. Segregation ratios for RAPD marker classes were tested for the expected 3:1 with chi-square test.

Results and Discussion

Phenotying of parents

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, 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 was illustrated as histogram [Figure 1].

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 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 the 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 is a moderate yielder with 45 quintals / hectare; superior grain quality for cooking purpose and it is widely being 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 might favour the variety to sustain yield losses.

Moroberekan is an upland japonica rice cultivar with 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 (RIL) population.

Phenotypic traits and their variations in F_2 mapping population

The F_2 families exhibited a wider variation from the parents for all the traits studied. Wider variation among the F_2 families for plant height was observed as it ranged from 48.00 cm to 125 cm with a mean height of 81.66 cm. Days to flowering among the F_2 individuals ranged from 71 days to 126 days with a mean of 99.78 days. Panicle length ranged from 11.00 cm to 27.00 cm with the mean length of

20.59 cm. Grain yield per plant ranged from 19.54 g 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 1.

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 subjected to artificial blast screening nursery (Figure 2) (Table 2). Disease 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. 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 more than two resistance genes present in the resistant parent.

Parental survey, Constitution of Bulks and BSA

In parental survey, out of 266 primers, 200 primers showed polymorphism between parents. A total of sixteen kits of RAPD Operon primers were tested for parental polymorphism. The genomic DNA of the selected F_2 individuals was used in BSA. Equal quantities of DNA from the selected 10 resistant F_2 individuals and 10 susceptible F_2 individuals were taken and pooled separately constituted the resistance bulk and susceptible bulk respectively (Table 3).

RAPD markers are often used in conjunction with bulk segregant analysis and detailed genetic maps, provide a very efficient method of characterizing and locating natural and induced mutated alleles at genes controlling interesting agricultural traits. The usual method to locate and compare loci regulating quantitative traits (QTLs) requires a segregating population of plants with each one genotyped with molecular markers. However, plants from such segregating populations can also be grouped according to phenotypic expression of a trait and tested for differences in allele frequency between the population bulks and this is Bulk Segregant Analysis (BSA) (Michelmore *et al*., 1991; Quarrie *et al*., 1999).

In this study of using BSA, twenty polymorphic primers produced a total of 291 fragments *viz.,* 152 amplicons produced by the resistant bulk and 139 amplicons generated by the susceptible bulk, out of which 23 fragments (8.74 per cent) were polymorphic and rest were monomorphic. The banding pattern in the resistant phenotype specific individual RAPD primers in bulk segregant analysis showed that OPAL 16 is a resistant phenotype specific RAPD primer. The RAPD primer OPAL 16 produced a resistant phenotype specific RAPD marker OPAL 16_{940}. The 940 bp fragment was present in the resistant parent and resistant bulk and was absent in the susceptible parent and bulk. Likewise, the following primers produced resistant phenotypic specific markers which are as follows: OPM 9_{350}, OPI 11_{420}, OPBD $12_{600,\ 680}$, OPAL 18_{1054}, OPM 15_{210}, OPBB 13_{755}, OPBB 5_{194}, OPBA 15_{458}, OPE 1_{947} and OPM 18_{697} (Table 4).

Co-segregation analysis

Co-segregation analysis was performed with the DNA of individual resistant and susceptible genotypes which were used to constitute the resistant and susceptible bulks. All the 11 RAPD primers showed dominant type of banding pattern. They amplified 12 resistant phenotype specific markers. The co-segregation banding of the RAPD primer OPBB5_{194}, OPBB13_{755}, and OPBD$12_{600,\ 680}$ in the resistant and susceptible F_2 individuals was shown in Fig.3. RAPDs are commonly inherited as dominant markers, where the presence of a particular band is dominant, and its absence is recessive (Tingley and Tufo, 1993). Linkage between a polymorphic marker and the target locus is confirmed and quantified by using the segregating population from which the bulks were generated. When the bulks differing for disease resistance were made and analyzed with markers known to be linked to the resistance gene, a clear polymorphism between the bulks comparable to that between the parents was observed (Michelmore *et al*., 1991).

Poulson *et al.* (1995) suggested that when bulks are constructed from enough individuals, the BSA is sufficiently robust to cope with the low level of

phenotypic misclassification. All polymorphic loci have been assayed with 15cM of the target locus are likely to be identified by BSA. This strategy has the advantage of identification of markers linked to the target residue (Chague *et al*., 1996). In this study, the identified RAPD markers are supposed to be linked very closely for blast disease resistance and other associated traits.

Mapping survey of the F_2 segregating population by RAPD markers and SMA

Among the eleven RAPD primers which co-segregated in the BSA, 7 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 4 RAPD primers *viz.,* OPE 1, OPI 11, OPM 15 and OPM 18. Out of 7 RAPD primers, 12 RAPD markers were scorable and none of the markers deviated from the expected Mendelian segregation ratio of 3:1, except OPBB 5_{258} which skewed more towards Moreoberekan (Table 5). 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 dominant resistant alleles (Moroberekan) rather than the recessive alleles (White Ponni). The banding profile of part of individual F_2 lines using OPBB $5_{700, 258}$ and OPBB 5_{194} marker segregation along with the parents White Ponni (P_1) / Moroberekan (P_2) are shown in Fig. 4.

In this study, an attempt was made to establish the genotype-phenotype association involving RAPD markers with phenotypic traits. The QTL analysis for all the six phenotypic characters was carried out using the Single Marker Analysis (SMA). One way ANOVA performed on the mean of groups formed based on the individual segregation pattern of 12 RAPD markers. A simple regression analysis was also performed by keeping the phenotypic value as the dependent variable and the individual segregation pattern of RAPD marker as independent variable. 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}) and 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. The details of the markers linked, the regression (R^2 per cent) values and the probability values are given in Table 6.

The single marker analysis (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 Single Marker Analysis can be implemented as a simple ANOVA, linear regression, t-test and maximum likelihood estimation (Haley and Knott, 1992; Neinhuis *et al*., 1987; Wang *et al*., 1994). 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.

At present, the phenotypic screening for blast has its own limitation as the exchange of material 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). The major hurdle in understanding the quantitative resistance to variety of diseases is the non-availability of the reliable screening methodologies and tediousness and complexity in the available methods. The present study could be a little step forward to achieve a path breaking approach for disease resistance in rice and will be helpful in future. In future attempts will be done to tag the resistance genes with identified markers, flanking markers will be cloned and sequenced [SCAR markers]. The closely linked markers will be checked with the mapping population.

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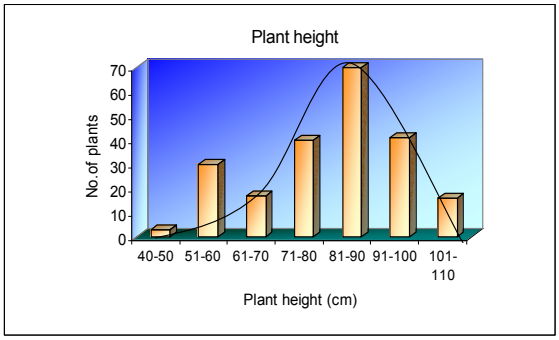

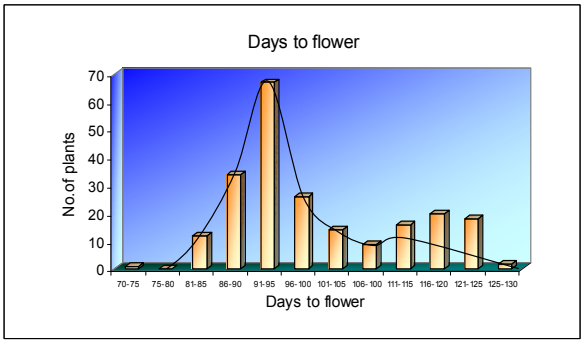

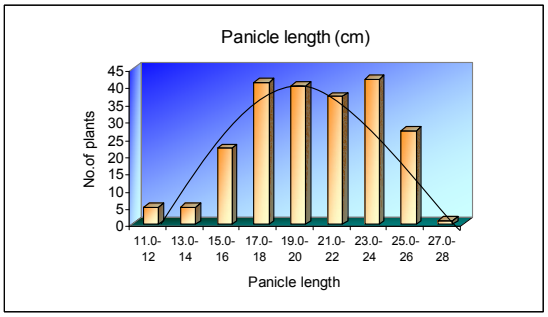

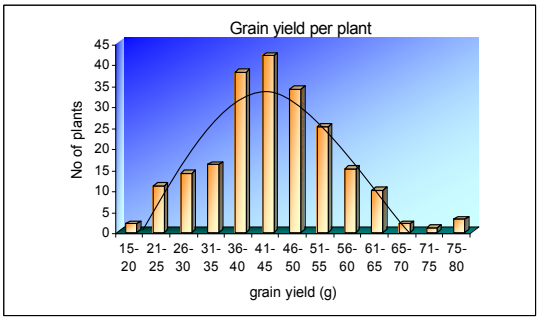

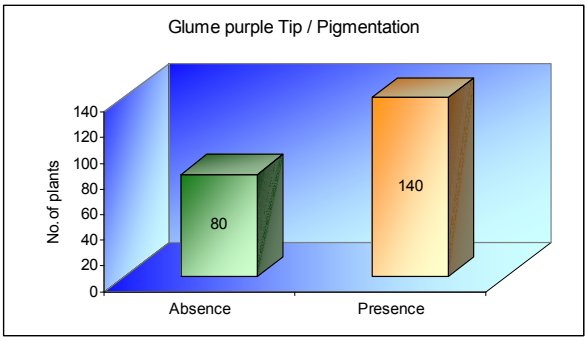

Fig 1(a). Frequency distribution of phenotypic traits over the F_2 populations of Whte ponni / Moroberekan

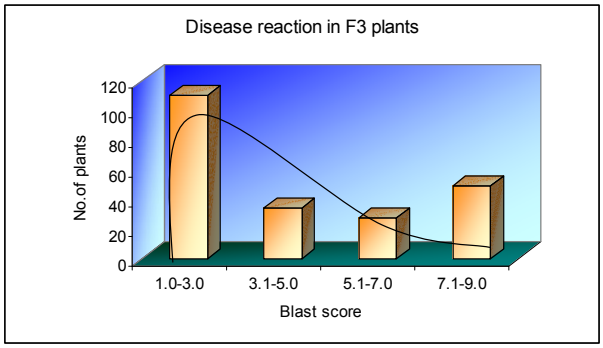

Fig 2. Frequency distribution of Blast disease score over the 220 F_3 families of White ponni x Moroberekan

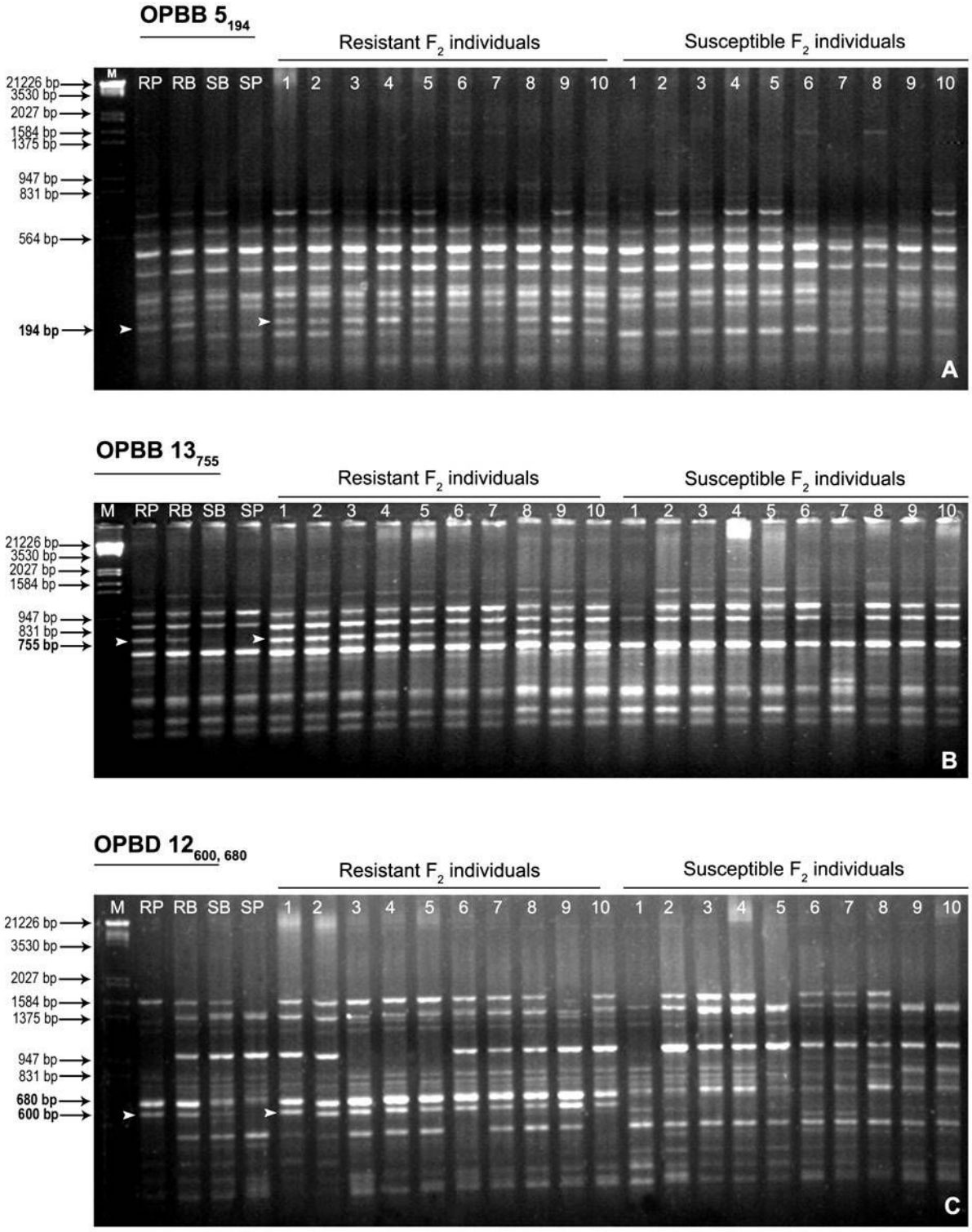

Figure 3 (A,B,C) Co segregation banding pattern of RAPD primers in the resistant and susceptible F_2 individuals

M - Marker; Lambda DNA/*Eco*RI+*Hin*dIII double digest, RB; Resistant bulk, SB; Susceptible bulk, RP; Resistant parent (Moroberekan), SP; Susceptible parent (White ponni),

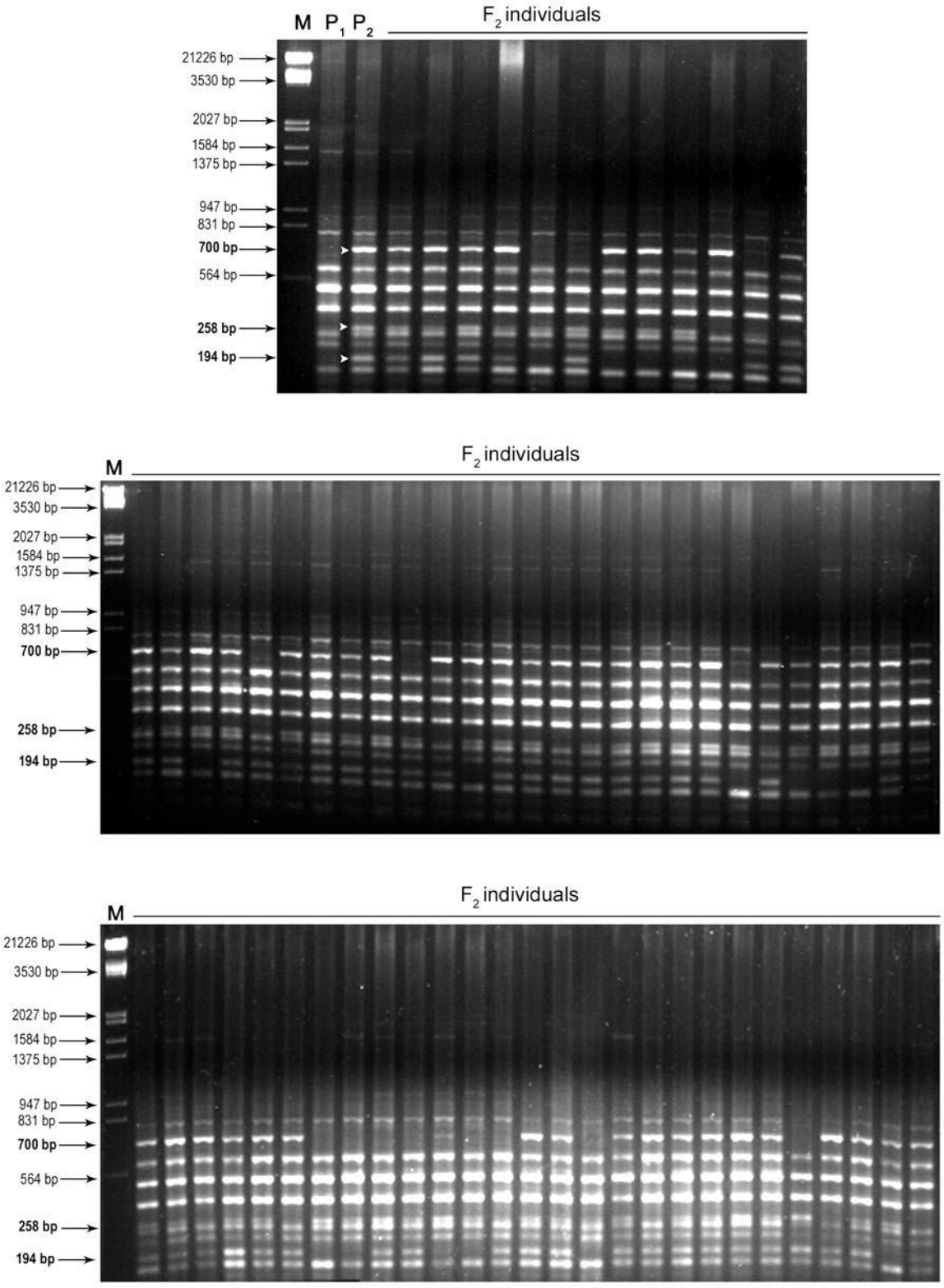

Figure 4. OPBB $5_{700, 258}$ and OPBB 5_{194} marker segregation among the individual F_2 lines of White Ponni (P_1) / Moroberekan (P_2)

M - Marker; Lambda DNA / *Eco*RI+*Hin*dIII, P_1 - White Ponni, P_2 - Moroberekan

Table 1. Variation for phenological traits among the $F_{2:3}$ mapping population of White Ponni / Moroberekan

Morphological traits/ Disease reaction	White Ponni (P_1)	Moroberekan (P_2)	Mean	Range	Sample Variance	Standard Error	Standard Deviation	Confidence (5 % / 1 %)
F_2 mapping population								
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 (g)	53.80**	39.90**	45.81**	19.54 – 100.26	198.03	0.95	14.07	1.870 / 2.470
F_3 population								
Leaf blast score (1 – 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 (Scored '1' for absence; '3' if pigmentation is present)

Table 2. 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

Table 2. (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)

P_1 – Female parent (White Ponni);
P_2; Male parent (Moroberekan)

Table 3. Resistant and susceptible F_2 individuals selected based on leaf blast disease scores obtained from corresponding F_3 families for bulk segregant analysis

Resistant F_2 Plant Number	Corresponding Mean PDI % of (F_3) family	Corresponding Mean Score of (F_3) family	Sample variance (SV)	Susceptible F_2 Plant number	Corresponding Mean PDI % of (F_3) family	Corresponding Mean Score of (F_3) family
1	27.04	2.43**	0.93	55	99.99	9.00
2	16.30	1.47**	0.71	69	99.99	9.00
3	19.63	1.85**	1.22	83	99.99	9.00
21	20.74	1.87**	1.17	144	99.99	9.00
31	21.85	1.97**	0.59	155	99.99	9.00
34	18.52	1.67**	1.66	167	99.99	9.00
41	23.70	2.13**	0.73	169	99.99	9.00
59	26.11	2.35**	1.06	197	99.99	9.00
61	16.30	1.47**	0.70	204	99.99	9.00
68	25.19	2.27**	0.85	206	99.99	9.00

R - Resistant – (Score 1.0 to 3.0)
MR - Moderately Resistant (Score 3.1 – 5.0)
MS – Moderately Susceptible (Score 5.1 – 7.0)
S – Susceptible (Score 7.1 – 9.0)
(SES, 2002)

Table 4. Details of RAPD markers amplifying resistant phenotype specific fragments in bulk segregant analysis

S.No	RAPD primer	RAPD marker phenotype	Primer sequence (5'-3')
1	OPE 1_{947}	Resistant	CCCAAGGTCC
2	OPI 11_{420}	Resistant	ACATGCCGTG
3	OPM 9_{350}	Resistant	GTCTTGCGGA
4	OPM 15_{210}	Resistant	GACCTACCAC
5	OPM 18_{697}	Resistant	CACCATCCGT
6	OPAL 16_{940}	Resistant	CTTTCGAGGG
7	OPAL 18_{1054}	Resistant	GGAGTGGACT
8	OPBA 15_{458}	Resistant	GAAGACCTGG
9	OPBB 5_{194}	Resistant	GGGCCGAACA
10	OPBB 13_{755}	Resistant	CTTCGGTGTG
11	OPBD $12_{600, 680}$	Resistant	GGGAACCGTC

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

S.No	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**

(χ2 value: 3.84 at $P \leq 0.05$ and 6.63 at $P \leq 0.01$)
- Deviation of the marker from Mendelian segregation ratio 3:1

Table 6. Summary of RAPD markers putatively associated with phenotypic traits identified by single marker analysis and simple regression analysis.

Markers	F(Cal)	Pr>F	R^2 (%)
Markers linked to leaf blast resistance			
OPBB 5 (258 bp)	14.993**	0.0001	6.40
OPBB 5 (194 bp)	8.764**	0.003	3.90
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			
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
OPBD 12 (600 bp)	4.235*	0.041	1.90
OPBB 13 (755 bp)	4.220*	0.041	1.90
Markers linked to grain yield per plant			
OPAL 16 (940 bp)#	7.909**	0.005	3.50

F-Critical value: 5% level – 3.037*; 1% level – 4.704**.
- Markers linked for more than one trait.