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Research Article

Genetic diversity analysis aiding in selection of parents by RAPD markers in rice (*Oryza sativa* L)

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Abstract:
Genetic diversity among 26 rice genotypes was investigated using RAPD markers. The genotypes were screened for the leaf blast disease reaction at two different environments. The average number of alleles amplified per primer was 9.03. Average number of polymorphic bands per primer was 6.80 with average polymorphism information content (PIC) of 0.264. Clustering based on dendrogram revealed two major clusters and 5 sub clusters. Principal Coordinate Analysis (PCoA) revealed three major groups. The first coordinate does not discriminate any of the genotypes based on the geographical origin, but the second and third coordinates differentiated South East Asian and South Asian genotypes clearly. Genetic diversity analysis of rice genotypes with RAPD marker system and phenotypic screening for blast resistance revealed that White Ponni (susceptible) and Moroberekan (resistant) were one among the genetically distant and contrasting parents for leaf blast resistance. There is no clear discrimination of the markers to distinguish leaf blast resistant and susceptible genotypes into separate clusters by the principal coordinate analysis.

Key words: Rice, Genetic Diversity, leaf blast resistance, *Magnaporthe grisea,* mapping population

Introduction

Rice is the primary food for more than three billion people around the world, providing the staple diet of more than half of the world's population. The estimated doubling of the population by 2050 will require a similar increase in food production (Maclean, 2002). This has to be achieved by the development of high yielding rice varieties with improved nutritional quality and tolerance to biotic and abiotic stresses. In addition, by increasing yields on land already in production, hundreds of millions of hectares of tropical forests and other natural environments were saved from conversion to agriculture (Toenniessen *et al*. 2003). Unfortunately, these expectations are short lived because the large areas of high yielding but genetically identical cultivars proved to be susceptible to pest and diseases. Among the biotic stresses, diseases continue to be the major threat for increased production. Hence, the most urgent need is to increase the yield of rice by managing the problems caused by biotic and abiotic stresses.

Nowadays, modern molecular marker technological tools are available to plant breeders and pathologists which offer several new possibilities to manifest the ill effects caused by various major disease causing pathogens resulting in severe yield losses. The possible ways to counter such yield losses is either identification of resistant varieties available in nature without compromising the yield or by incorporating combination of major resistance genes in high yielding varieties to increase productivity and crop diversification, while developing a more sustainable agriculture. The other way is by elucidating the basis of plant resistance through a comprehensive analysis of the molecular events that occur during pathogen-host recognition and the subsequent defense responses.

Plant biotechnology applications must not only respond to the challenge of improving food security and fostering socio-economic development, but in doing so, promote the conservation, diversification and sustainable use of plant genetic resources for food and agriculture. The narrow genetic base of rice

(*Oryza sativa* L.) cultivars poses a challenge for long-term improvements of yield and susceptibility of the genotypes to major diseases. Molecular marker analysis can be used to quantify the divergence and similarity of rice genotypes based on which rational strategies can be adopted for the selection of suitable entries with broader genetic base and desirable traits to incorporate them in future breeding programs (Hittalmani *et al*, 2000).

Knowledge of genetic diversity present within a species is a pre-requisite for the development of mapping population by selecting the suitable parents with broad genetic base and greater amount of divergence between the two genotypes. Genetic diversity studies employing various molecular markers at DNA level in combination with the morphological traits of the selected genotypes enable breeders to formulate successful hybridization programmes.

The rice blast disease caused by *Magnaporthe grisea* (Hebert) Barr. (Asexual form known as *Pyricularia grisea* (Cooke) Sacc.), is one of the most serious fungal diseases which are widespread threatening the world rice production (Ou, 1985). Genetic resistance to rice blast has been and continues to be extensively used by rice breeders and pathologists to combat this disease. 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, 2006).

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).

In RAPD technique, DNA polymorphisms are produced by "rearrangements or deletions at or between oligo-nucleotide primer binding sites in the genome" (Welsh and McClelland, 1990; Williams *et al*. 1990) as it provides a convenient and rapid assessment of the differences in the genetic composition of the related individuals. With the help of RAPD, genetic variations have been detected, both, within and between species of plants (Bautista *et al*. 2006; Kwon *et al*. 2002; Ravi *et al*. 2003; Qian *et al*. 2006; Khandelwal *et al*. 2005; Ishii *et al*. 2006; Vanaja *et al*. 2006). In the light of the above facts and considering the potentials of DNA markers, the present study was undertaken with the following objectives: 1) to assess the genetic diversity existing in the rice genotypes through molecular markers. 2) to screen the rice genotypes for leaf blast disease reaction at two environments and 3) to compare the disease reaction pattern with the genetic diversity results and 4) to select the blast resistant and susceptible parent for effecting hybridization and development of mapping population.

Material and methods:

A] Plant material:

Twenty six cultivars of rice *Oryza sativa* L., from different geographical origin, commonly used as the parents in programmes aimed at developing high-yielding hybrids with blast resistance were selected for this study (Table 1). These genotypes were obtained from Paddy Breeding Station, Coimbatore and Central Rice Research Institute (CRRI), Cuttack in the year 2005, which includes 6 ARBN lines (Asian Rice Biotechnological Network) introgressed with leaf blast disease resistance genes.

B] Field screening for leaf blast disease reaction

All the rice genotypes were screened at Hybrid Rice Evaluation Centre, Gudalur, Tamilnadu, India (hot spot for leaf blast), where disease occurrence is throughout the year and maximum during winter season. Each entry was sown in a single row and replicated thrice with every adjacent row planted with Bharti, (a highly susceptible local cultivar for leaf blast). The entire nursery was surrounded on all sides by two rows of Bharti, as a spreader source for the pathogen. The observation of disease reaction was recorded, when the susceptible check was severely infected by leaf blast.

Individual plant in each entry was scored based on the leaf blast severity following Standard Evaluation System (SES, IRRI, 2002) on a 0-9 scale as detailed at 35th day after sowing, when the susceptible check (Bharti) was fully infected. The Potential Disease Incidence (PDI %) per cent was worked out using the formula given by McKinney (1923) :

PDI % = (Sum of numerical rating / Number of leaves observed) x (100 / Maximum disease score).

b) Artificial screening for leaf blast disease reaction:

Artificial screening for rice blast disease was done in the 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 per cent 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 entry following Standard Evaluation System (SES, IRRI, 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.

DNA extraction

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). DNA was checked for its purity and intactness and then quantified. The crude genomic DNA was run on a 0.8 per cent agarose gel stained with ethidium bromide following the protocol of Sambrook *et al.* (1989) and was visualized in a gel documentation system (Alpha Imager ^{TM}1200, Alpha Innotech Corp., California, USA). Intact and pure genomic DNA was assessed with agarose gel electrophoresis. Then, it was quantified with flourimeter (DyNA Quant^{TM}200, Hoefer, CA, USA). Based on the quantification data, DNA dilutions were made in 1 X TE buffer for a volume of 250 µl (working solution) to a final concentration of 15 ng per µl and stored in 4° C.

Molecular marker assay:

Twenty six rice genotypes were used for this study. RAPD analysis was carried out on these genotypes at Molecular Marker Assisted Selection Laboratory, Dept. of Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, India. A total of 53 decamer primers supplied by Operon Technologies Inc., Alameda, California, USA were used in the study of genetic diversity analysis for 26 rice genotypes after screening randomly chosen five varieties using 120 RAPD primers. Out of 53 primers used to amplify twenty six rice genotypes, only 36 primers generated clear banding pattern. Amplification reactions were in volumes of 20 μl containing 10 mM Tris HCl (pH 9), 50 mM KCl, 1.5 mM MgCl2, 0.001 per cent gelatin, dATP, dCTP, dTTP and dGTP (each at 0.1 mM), 0.2 mM primer, 25–30 ng of genomic DNA and 0.3 unit of *Taq* DNA polymerase. Amplifications were performed in 96 well thin wall polycarbonate microtitre plates (Corning Inc.) in a PTC 100 Thermal cycler (MJ Research Inc.) programmed for 35 cycles of 1 min at 92 ◦C, 1 min at 36 ◦C and 2 min at 72 ◦C preceded and followed by 2 min at 92 ◦C and 10 min at 72 ◦C respectively. PCR Amplified products (15μl) were subjected to electrophoresis in 1.5 per cent agarose gels in 1X TBE buffer at 60V for 1 h using Bio-Rad® submarine electrophoresis unit. The electronic image of the Ethidium bromide stained gel was visualized and documented in a gel documentation system (Alpha Imager ^{TM}1200, Alpha Innotech Corp., California, USA).

Data analysis:

Scoring of RAPD bands was carried out by considering only the clear and unambiguous bands. Markers were scored for the presence and absence of the corresponding band among the different genotypes. The scores '1' and '0' were given for the presence and absence of bands, respectively. Polymorphism information content (PIC) or expected heterozygosity scores for each RAPD markers were calculated based on the formula, $Hn = 1 - \Sigma pi^2$, where pi is the frequency for the i-th allele (Nei, 1973). The data obtained by scoring the RAPD profiles of different primers were subjected to cluster analysis. Similarity matrices constructed using Jaccard's coefficient were used for sequential agglomerative hierarchical non-overlapping (SAHN) clustering based on the unweighted pair group method with arithmetic averages (UPGMA), using NTSYSpc version 2.02 (Rohlf, 2000).

Results and Discussion:

Among the genotypes screened, highly significant lower mean disease reaction score (2.30 and 0.84) and mean PDI (Potential Disease Incidence) per cent (25.25 and 9.33) was recorded by Moroberekan in natural and artificial screening respectively. The higher mean disease reaction score and mean PDI % was recorded by IR 50 (7.79 and 87.78 %) followed by White Ponni (7.52 and 83.54 %) under natural conditions. Higher mean disease reaction scores was recorded by TN 1 (8.60 and 95.55 %) followed by White Ponni (8.50 and 94.50), under artificial conditions (Table 2 and Table 3).

Among fifty three random primers used in this study, thirty six primers detected a total of 325 amplicons in twenty six genotypes, out of which 245 were polymorphic. The number of primers used in this experiment was sufficient enough to characterize the genotypes, as previously the number of RAPD primers used was 36 primers for 40 genotypes of rice (Ravi *et al*. 2003), 43 primers for 13 genotypes of rice (Kwon *et al*. 2002), 10 primers for 18 genotypes of rice (Raghunathachari *et al*. 2000). The total number of markers varied from 4 to 17 with a mean of 9.03 markers per primer (Figure 1).

Marker Index (MI) reveals the amount of information that can be obtained from a particular primer. Higher the MI, more the informativeness of the primer. The marker index among the RAPD primers ranged from 0.336 to 7.378 in this analysis. The abstract of the level of polymorphism detected among the genotypes are listed in Table 4. PIC values are dependent on the genetic diversity of the genotypes chosen (Manimekalai and Nagarajan, 2006). PIC provides an estimate of the discriminating power of the marker. This was evident in the present study too, as the highest PIC value was observed for the primer OPM 4 (0.434). The PIC values ranged from 0.137 to 0. 434, which was in accordance to the results obtained by Hongtrakul *et al*. (1997) with 0.0 to 0.500, Manimekalai and Nagarajan (2006) with 0.031 to 0.392. The number of polymorphic markers for each primer varied from 2 to 17 with a mean of 6.80 polymorphic markers per primer (Table 5).

Jaccard's coefficient of similarity ranged from 0.470 to 0.839 with a mean of 0.640 (Table 6). Most of the pair-wise similarity values fell into the range of 0.601 – 0.700. The genotypes Tadukan and ARBN 97 were closest in the study with a genetic similarity value of 0.839 followed by CB 98013 and ARBN 139 with a value of 0.787. The genotypes BPT 5204 and CB 98006 had the lowest similarity index of 0.470. In the present investigation, the mean Jaccard's similarity value was calculated for the genotypes belonging to the different geographic regions to know the similarity level among the genotypes within the geographic region. The highest mean similarity value was noticed among the South East Asian genotypes (0.664) followed by South Asia / African genotypes (0.646) and South Asian genotypes (0.604) based on RAPD markers. Presence of high diversity among the South Asian genotypes arrived from this study suggests that India as one of the major centres of diversity notably the mid-Eastern part and the North Eastern hills as indicated by Sarla *et al*. (2005).

The dendrogram revealed two major clusters, Cluster **1** and Cluster **2** which was further divided to five sub-clusters (figure 2). Cluster **1a** consisted of 8 genotypes of which four belonged to South East Asia (TN 1, ADT 43, IR 64 and Tadukan), one each from South East / South Asia (CO 43), South Asia (CB 98013) and two genotypes (ARBN 97, ARBN 139) from (South Asia / Africa). Cluster **1b** consisted of three accessions, each from South East Asia (Milyang 46), Central Asia (ARBN 153) and from South Asia (Ajaya). Cluster **1c** revealed 5 genotypes two each from South East Asia (ARBN 138, Tetep) and South Asia (BPT 5204 and Pusa Basmati) and one from Africa (Moroberekan). Cluster **1d** consisted of 4 genotypes of which two belonged to South East Asia (ARBN 142 and IR 36) and each one from South Asia (CB 98004) and Latin America (Columbia – 2). Cluster **1e** consisted of 3 genotypes of which two belonged to South East Asia (White Ponni and IR 50) and one genotype from South Asia / African origin. Cluster **2** consisted of 3 genotypes; all three are from South Asia (CB 98002, CB 98006 and ASD 16).

Majority of the clustering patterns from the dendrogram showed that the South East Asian genotypes clustered along with the South Asian genotypes except the major cluster '2' consisted all of three South Asian varieties and it might be due to the adaptation of the cultivars to the prevailing ecological and climatic conditions as pointed out by many scientists. Sun *et al*. (1999) observed similar results in their investigation, where the RAPD band sharing data which showed no correlation with the geographic origin and the clustering pattern. They concluded that geographically close habitats might be ecologically quiet different and conversely, habitats that are geographically distant from one another can be very similar in their environmental conditions.

The extensively used hierarchical methods, such as UPGMA, might not be appropriate for the clustering of genotypes if the materials studied were of intra-specific in nature. Hence, Principal Coordinate Analysis might be appropriate (Chaparro *et al*, 2004). Applying both methods was recommended to extract the maximum amount of information from the molecular (matrix) data (Messmer *et al*, 1992). Clustering was useful in detecting relationships among lines, while Principal Coordinate Analysis allowed a view on the relationships between groups.

Principal coordinate analysis (PCoA) resulted in a two dimensional scatter plot which revealed three major groups of accessions belonging to South East Asia and South Asia in group I, all three South Asian varieties in Group III and Group II consisted of all South East Asian varieties except a Latin American variety and a Basmati genotype from India. The three principal coordinates (PCo1, PCo2 and PCo3) encompassed 89.27 per cent, 6.07 per cent and 2.72 per cent of variation respectively (Figure 3).

There is no clear discrimination of the RAPD markers to distinguish leaf blast resistant and susceptible genotypes into separate coordinates by the Principal Coordinate Analysis. For the success of any breeding program, it is essential to know the variability in the disease expression of the resistant and susceptible parents under varying environmental conditions and to know their genetic constituents (Padmanabhan *et al*. 1973). It is also inevitable to screen the parental materials under prevailing environmental conditions of specific location with at least the strain or isolate of that location where breeding programmes like hybridization, development of mapping populations are being done. 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 (Atlin et al., 2004). Therefore, emphasis was given to choose appropriate parents in order to obtain useful segregants.

The selection of suitable parents for the constitution of mapping population was done based on the results obtained from the genetic diversity analysis using the RAPD marker system and the leaf blast disease reaction of the rice genotypes studied. The results based on the diversity analysis indicated that the genotypes, White Ponni and Moroberekan were present in different clusters based on the dendrogram. The genotype Moroberekan was found in the sub cluster '1b' and White Ponni was located in the sub cluster '1e' as evident that both the genotypes were divergent in nature. The two dimensional scatter plot generated by the Principal Coordinate Analysis (PCoA) also indicated that both the genotypes were present in two different groups. The genotype, Moroberekan was located in the 'Group I' and White Ponni was located in the 'Group II' of the scatter plot diagram. Similar kind of selection based on the dendrogram was done by selecting wheat genotypes, Kharchia 65 and TW 161 as parents for mapping population to map QTLs for saline tolerance. They were genetically distant (similarity coefficient 0.54) from each other and they were located in two different clusters (Shazad and Salam, 2006).

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Table 1. Genotype details and their geographic origin of the rice genotypes used in this study

Genotypes	Pedigree	Habit	Duration (days)	Place of collection	Geographic origin
Ajaya	IET 4141 / CR 987216	Semi dwarf	105	India	South Asia
ASD 16	ADT 39 / CO 39	Semi dwarf	110-115	India	South Asia
BPT 5204	GEB-24 / T(N) 1 / Mahsuri	Semi dwarf	140-145	India	South Asia
CB 98002	TNAU 89093 / ASD 5	Semi dwarf	130	India	South Asia
CB 98004	TNAU 89093 / ADT 40	Semi dwarf	130	India	South Asia
CB 98006	Ponni / CO 43	Semi dwarf	135	India	South Asia
CB 98013	CO 45 / IR 64	Semi dwarf	138	India	South Asia
Pusa Basmati	Pusa 167 / Karnal local	Semi dwarf	115	India	South Asia
IR 50	IR 2153-14 / IR 28 / IR 36	Dwarf	115	Philippines	South East Asia
ARBN 138	*Oryza minuta* (Acc. 10114) / (WHD-IS-1-127) / (DM 360)	Dwarf	135	Philippines	South East Asia
ARBN 142	BL 142	Semi dwarf	130	Philippines	South East Asia
IR 36	IR 1561-228 // IR 24 4 / *O. nivara* // CR 94-13.	Dwarf	110	Philippines	South East Asia
IR 64	IR 5657-3-3-3-1 / IR 2061-465-1	Semi dwarf	115-120	Philippines	South East Asia
Milyang 46	Doosan 8 / Sacheon 8	Dwarf	110	South Korea	South East Asia
Tadukan	Philippine *indica* cultivar (Luzon)	Semi dwarf	130-135	Philippines	South East Asia
Tetep	Vietnamese *indica* cultivar	Semi dwarf	130-135	Vietnam	South East Asia
TN 1	Chow-Woo-Gen / Tsai-Yuan-Chung.	Dwarf	120-125	Taiwan	South East Asia
White Ponni	Taichung 65/2 / Mayang Ebos- 80	Tall	125-130	Malaysia	South East Asia
ADT 43	IR 50 / Improved White ponni	Semi dwarf	110	India	South / S.E. Asia
CO 43	Dasal / IR 20	Dwarf	130-135	India	South / S.E. Asia
ARBN 153	C-101-Pai Kan Too (*japonica*)	Tall	110-115	China	Central Asia
ARBN 97	RIL 45 (Moroberekan / CO 39)	Semi dwarf	135	India	South Asia / Africa
ARBN 139	RIL 10 (Moroberekan / CO 39)	Dwarf	140	India	South Asia / Africa
ARBN 144	RIL 249 (Moroberekan / CO 39)	Semi dwarf	135	India	South Asia / Africa
Moroberekan	Guinean (West Africa) cultivar, *japonica*	Semi dwarf	130	Guinea (Africa)	Africa
Columbia - 2	Columbian *indica* cultivar	Semi dwarf	135	Columbia	Latin America

Source: (www.irri/iris/ htm) # - Online information collected from 'International Rice Information system'

Table 2. Rice blast disease reaction at HRE, Gudalur (Field screening).

Genotypes	Mean disease Score	Mean PDI (%)	Blast disease reaction	Standard error	Standard deviation	Sample variance	Significance (5% / 1 %)
ARBN 97	2.78**	30.96	R	0.340	1.701	2.893	0.702 / 0.941
ARBN 138	2.57**	28.59	R	0.595	2.972	6.840	1.227 / 1.730
ARBN 139	2.36**	26.22	R	0.270	1.352	1.827	0.558 / 0.791
ARBN 142	3.30**	36.74	MR	0.574	2.868	5.227	1.184 / 1.655
ARBN 144	6.05**	67.25	MS	0.432	2.160	4.667	0.892 / 1.265
ARBN 153	2.52**	27.99	R	0.623	3.113	6.663	1.285 / 1.782
IR 64	0.60*	6.67	R	0.208	1.041	1.083	0.438 / 0.805
CB 98002	3.48**	38.66	MR	0.530	2.651	7.027	1.094 / 1.546
CB 98004	3.10**	34.51	MR	0.399	1.993	3.973	0.823 / 1.137
CB 98006	5.10**	58.58	MR	0.494	2.471	6.107	1.020 / 1.446
CB 98013	0.60*	6.67	R	0.329	1.645	2.707	0.438 / 0.805
Columbia 2	0.30*	3.33	R	0.115	0.577	0.333	0.238 / 0.334
Moroberekan	2.30**	25.57	R	0.383	1.915	3.667	0.790 / 1.104
Milyang 46	2.57**	28.59	R	0.462	2.309	5.333	0.953 / 1.308
Tadukan	0.50	5.56	R	0.673	3.367	6.333	1.370 / 1.896
Tetep	0.33	3.39	R	0.374	1.869	3.493	0.772 / 1.069
IR 50	7.79**	87.78	S	0.360	1.523	2.333	0.631 / 0.882
TN 1	7.29**	81.33	S	0.503	2.517	6.333	1.309 / 1.444
White Ponni	7.52**	83.54	S	0.605	3.026	9.157	1.249 / 1.764
BPT 5204	7.07**	78.58	S	0.408	2.040	4.160	0.842 / 1.194
ADT 43	3.30**	36.74	MR	0.608	3.040	7.240	1.255 / 1.756
ASD 16	7.08**	78.66	S	0.346	1.732	3.00	0.715 / 1.00
CO 43	2.59**	28.77	R	0.400	2.01	4.35	0.826 / 1.167
Pusa Basmati	2.95**	32.77	R	0.562	2.812	5.907	1.161 / 1.644
Ajaya	5.18**	57.62	MS	0.364	1.818	3.037	0.751 / 1.055
IR 36	5.20**	57.72	MS	0.383	1.913	3.660	1.112 / 0.046

* - Significant at 5 % level; ** - Significant at 1 % level, (SES, 2002)

Blast disease reaction: 1- 3.0 = R, (Resistant), 3.1 – 5.0 = MR (Moderately Resistant), 5.1 – 7.0 = MS (Moderately Susceptible), 7.1 – 9.0 = S (Susceptible)

Table 3. Rice blast disease reaction at PBS, Coimbatore (Artificial screening)

Genotypes	Mean Disease Score	Mean PDI (%)	Blast disease reaction	Standard Error	Standard Deviation	Standard Variance	Significance (5 % / 1 %)
ARBN 97	7.02**	78.07	S	0.547	2.678	7.712	1.131 / 1.535
ARBN 138	6.74**	74.95	MS	0.564	2.671	7.623	1.666 / 1.582
ARBN 139	6.76**	75.10	MS	0.506	2.479	6.382	1.047 / 1.421
ARBN 142	0.88**	9.77	R	0.253	1.239	1.536	0.532 / 0.710
ARBN 144	1.77*	19.71	R	0.564	3.203	5.610	1.353 / 1.836
ARBN 153	7.56**	83.99	S	0.311	1.523	2.391	0.643 / 0.873
IR 64	0.61*	6.81	R	0.233	1.142	1.304	0.482 / 0.654
CB 98002	1.82**	20.29	R	0.560	2.745	7.536	1.159 / 1.573
CB 98004	5.20**	57.77	MS	0.425	2.083	4.341	0.880 / 1.194
CB 98006	6.09**	67.55	MR	0.333	1.633	2.667	0.690 / 0.937
CB 98013	1.38**	15.40	R	0.342	1.676	2.810	0.708 / 0.961
Columbia 2	1.06**	11.25	R	0.225	1.110	1.210	0.465 / 0.630
Moroberekan	0.84**	9.33	R	0.175	0.859	0.737	0.363 / 0.492
Milyang 46	1.17*	13.03	R	0.381	1.865	3.478	0.788 / 1.069
Tadukan	0.81*	9.03	R	0.451	2.212	4.895	0.634 / 0.831
Tetep	1.62**	18.07	R	0.590	2.889	3.348	1.220 / 1.601
IR 50	6.92**	76.88	S	0.419	2.053	4.216	0.867 / 1.177
TN 1	8.60**	95.55	S	0.359	1.761	3.101	0.744 / 1.009
White Ponni	8.50**	94.50	S	0.465	2.278	5.188	0.962 / 1.305
BPT 5204	8.25**	91.70	S	0.567	2.823	7.971	1.192 / 1.618
ADT 43	3.06**	34.06	R	0.491	2.408	5.797	1.017 / 1.380
ASD 16	7.21**	80.14	S	0.295	1.445	2.087	0.610 / 0.828
CO 43	1.85**	20.58	R	0.561	2.749	7.558	1.161 / 1.575
Pusa Basmati	1.17**	13.01	R	0.382	1.871	3.500	0.790 / 1.072
Ajaya	2.94**	32.73	R	0.282	1.382	1.911	0.584 / 0.792
IR 36	6.46**	71.84	MS	0.398	1.949	3.797	0.823 / 1.117

* - Significant at 5 % level; ** - Significant at 1 % level, (SES, 2002); Blast disease reaction: 1- 3.0 = R, (Resistant), 3.1 – 5.0 = MR (Moderately Resistant), 5.1 – 7.0 = MS (Moderately Susceptible), 7.1 – 9.0 = S (Susceptible)

Table 4. Level of polymorphism detected by RAPD markers among the rice genotypes

Parameters	Values
Number of primers used	53
Number of primers produced polymorphic amplicons	36
Total number of amplicons	325
Average amplicons per primer	9.03
Maximum number of amplicons by a single primer	17
Minimum number of amplicons by a single primer	4
Total number of polymorphic amplicons	245
Average polymorphic amplicons (%)	75.38
Maximum number of polymorphic amplicons by a single primer	17
Minimum number of polymorphic amplicons by a single primer	2
Average number of polymorphic amplicons per primer	6.80
Genetic similarity coefficients of all pairs of genotypes	
a) Maximum	0.839
b) Minimum	0.470
c) Average	0.640
Genetic distance (complement of Jaccard's coefficient)of all pairs of genotypes	
a) Maximum	0.530
b) Minimum	0.161
c) Average	0.360

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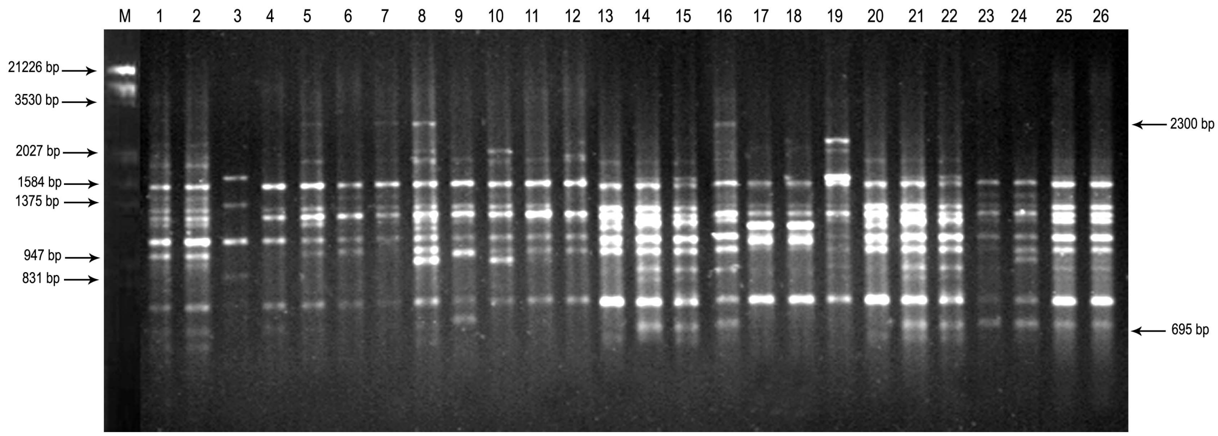
Table 5. Details of RAPD markers and their PIC and MI values

S.No	Primer	Total no of alleles	No of polymorphic alleles	Polymorphism (%)	Product size (bp)	PIC	MI
1	OPC 1	6	6	100.00	967-528	0.272	1.632
2	OPC 2	10	4	40.00	1204-389	0.294	1.176
3	OPC 3	12	7	58.33	1610-288	0.372	2.604
4	OPC 4	8	5	62.50	950-182	0.379	3.032
5	OPC 6	16	16	100.00	1913-325	0.394	6.304
6	OPC 16	7	6	85.71	1900-148	0.056	0.336
7	OPC 19	10	10	100.00	2124-690	0.342	2.736
8	OPE 1	8	5	62.50	2090-802	0.235	1.175
9	OPE 4	8	6	75.00	1380-330	0.216	1.296
10	OPE 16	6	4	66.67	978-148	0.278	1.112
11	OPE 18	5	2	40.00	920-110	0.191	0.382
12	OPE 20	10	8	80.00	1596-589	0.223	1.784
13	OPM 1	5	4	80.00	1380-178	0.272	1.088
14	OPM 4	17	17	100.00	2300-695	0.434	7.378
15	OPM 5	12	11	91.67	1380-103	0.277	3.047
16	OPM 8	7	2	28.57	850-160	0.156	0.312
17	OPM 9	6	5	83.33	1585-260	0.326	1.970
18	OPM 10	6	5	83.33	980-420	0.168	0.840
19	OPM 12	8	6	75.00	1178-178	0.305	1.525
20	OPM 13	5	5	100.00	1884-660	0.242	2.170
21	OPM 16	5	3	60.00	1188-158	0.227	0.681
22	OPM 17	4	3	75.00	1217-139	0.323	0.969
23	OPM 19	9	7	77.78	1420-368	0.253	1.711
24	OPN 2	11	10	90.91	1255-429	0.252	2.520
25	OPN 3	11	8	72.73	1204-106	0.243	1.944
26	OPU 14	9	6	66.67	1210-152	0.296	1.776
27	OPU 15	8	4	50.00	1295-126	0.137	2.192
28	OPBE 3	10	8	80.00	1580-589	0.245	3.430
29	OPBE 8	12	10	83.33	1645-128	0.252	2.520
30	OPBE 10	11	8	72.73	1480-330	0.231	1.848
31	OPBE 12	11	10	90.91	1375-330	0.221	2.431
32	OPBE 14	7	6	85.71	1375-570	0.386	2.702
33	OPBE 17	12	6	50.00	1187-128	0.211	1.266
34	OPBE 18	17	16	94.12	1344-116	0.220	3.520
35	OPBE 19	7	4	57.14	1129-83	0.261	1.044
36	OPBE 20	9	8	88.89	2850-620	0.311	2.498
	Total	325	245				
	Mean	9.03	6.80	75.38		**0.264**	**2.082**

Table 6. Similarity matrix among rice genotypes based on RAPD markers

GSIM	TN1	CB98013	A139	ADT43	IR64	CO43	AR97	TADN	1R50	MIL46	AJAY	A153	A144	MOBN	CB982	BPT	A138	TETP	WPON	CB986	ASD16	A142	CB984	IR36	COL2
TN1	1.0																								
CB93	0.8	1.0																							
A139	0.7	**0.8**	1.0																						
Adt43	0.7	0.7	0.7	1.0																					
IR64	0.7	0.8	0.7	0.8	1.0																				
CO43	0.6	0.7	0.7	0.7	0.7	1.0																			
AR97	0.7	0.8	0.7	0.8	0.8	0.8	1.0																		
Tadn	0.7	0.8	0.7	0.7	0.7	0.7	**0.8**	1.0																	
1R50	0.6	0.7	0.6	0.7	0.7	0.7	0.7	0.7	1.0																
Mil46	0.7	0.8	0.7	0.7	0.7	0.7	0.8	0.8	0.7	1.0															
AJAY	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.7	0.7	0.8	1.0														
A153	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.6	0.7	0.7	1.0													
A144	0.6	0.7	0.6	0.6	0.6	0.7	0.6	0.6	0.7	0.6	0.6	0.7	1.0												
MOBN	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.6	0.7	0.6	0.7	0.6	1.0											
CB982	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.7	0.6	1.0										
BPT5	0.6	0.7	0.7	0.7	0.6	0.6	0.7	0.7	0.6	0.7	0.7	0.7	0.6	0.7	0.6	1.0									
A138	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.6	0.7	0.6	0.7	0.6	0.7	0.6	0.7	1.0								
TETP	0.6	0.7	0.6	0.7	0.6	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.6	0.7	0.6	0.7	0.8	1.0							
WPON	0.6	0.6	0.6	0.7	0.6	0.6	0.7	0.7	0.6	0.7	0.7	0.7	0.5	**0.6**	0.5	0.6	0.7	0.7	1.0						
CB986	0.5	0.6	0.5	0.5	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.6	0.5	0.7	**0.5**	0.5	0.6	0.6	1.0					
Asd16	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.6	0.6	0.7	0.5	0.5	0.6	0.6	0.7	1.0				
A142	0.7	0.7	0.7	0.6	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	1.0			
CB984	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.6	0.7	0.6	0.7	0.5	0.6	0.6	0.6	0.6	0.5	0.6	0.7	1.0		
IR36	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.6	0.7	0.7	0.7	0.6	0.6	0.5	0.6	0.6	0.6	0.7	0.5	0.6	0.7	0.7	1.0	
COL2	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.5	0.6	0.5	0.6	0.6	0.6	0.6	0.5	0.5	0.7	0.7	0.7	1.0
PBAS	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.5	0.6	0.7	0.7	0.6	0.7



M – Marker, Lambda DNA / EcoRI + HinDIII double digest

Figure 1. Banding profile generated by OPM 4 for the rice genotypes

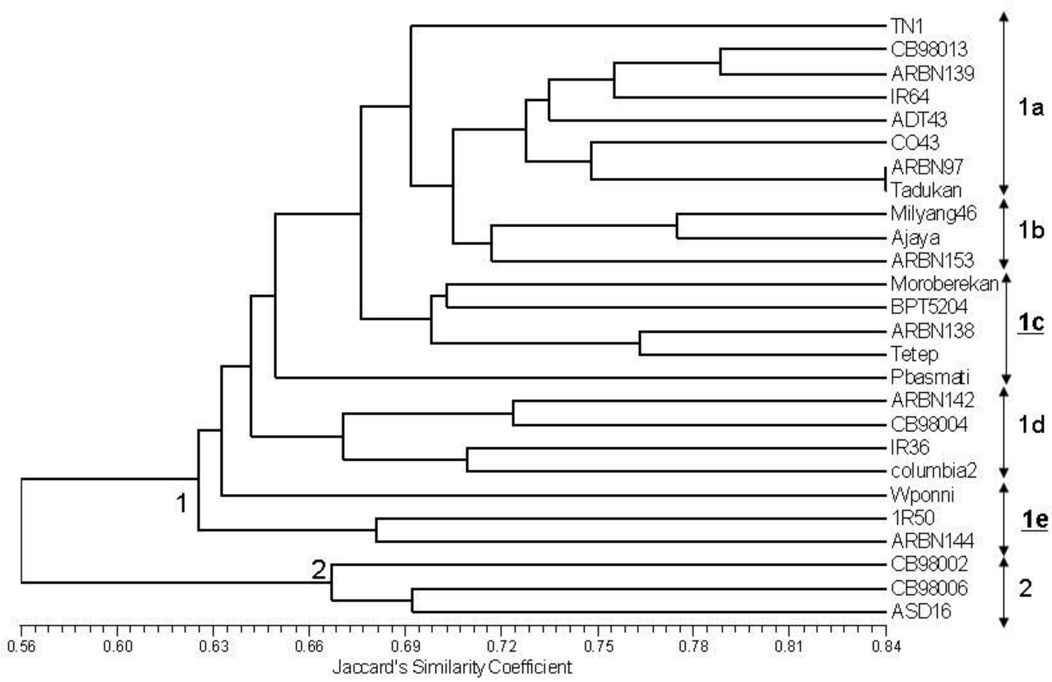


Figure 2. Phylogenetic analysis of rice genotypes based on RAPD markers

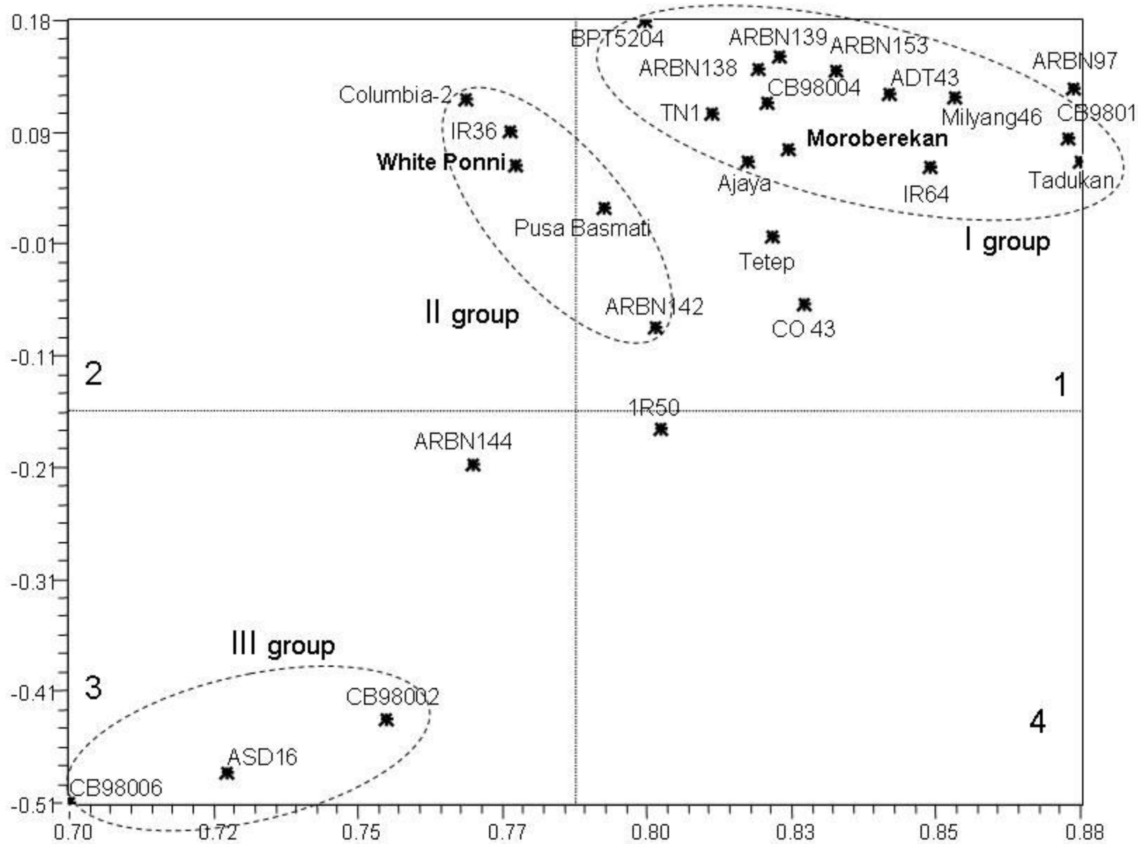


Figure 3. Principal Coordinate Analysis of rice genotypes based on RAPD markers