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Genetic diversity analysis aiding in selection of parents to generate mapping population revealed by molecular markers in rice (*Oryza sativa L.*)

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ABSTRACT

Genetic diversity among 26 rice genotypes from different geographical origin was investigated using molecular markers. The genotypes were screened for the leaf blast disease reaction at two different hot spot environments. The disease reaction scores were compared with the clustering pattern. The mean marker index (MI) for the primers was 2.082. The average pair wise similarity values based on their geographical origin revealed that the South East Asian genotypes had the highest value of 0.664, followed by (0.604) South Asian genotypes. Most of the pair wise comparisons fell into the similarity range of 0.601-0.700. Clustering based on dendrogram revealed two major clusters and 5 sub clusters. The South East Asian genotypes and South Asian genotypes clustered together in all branches of dendrogram indicating that both groups were diverse, except the major cluster '2' consisted all of three South Asian varieties. In Principal Coordinate Analysis (PCoA), the first three coordinates, accumulated 98.06 per cent of total variation. 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 genetically distant and contrasting parents for leaf blast resistance for mapping population development. There is a partial discrimination of the RAPD markers to distinguish leaf blast resistant and susceptible genotypes into separate clusters by the principal coordinate analysis.

KEYWORDS

Artificial screening;
Genetic Diversity;
Leaf blast disease;
Oryza Sativa;
Magnaporthe grisea;
Mapping polulation.

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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. This has to be achieved by the development of high yielding rice varieties with improved nutritional quality and tolerance to biotic and abiotic stresses. Asia's 'Green Revolution' achieved with increase in crop productivity that were sufficient to lower the proportion of population suffering from chronic hunger from 40 percent to 20 percent, while the overall population growth is more than doubled. 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[1]. 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 toolbox available to plant breeders and pathologists offers 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.

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. 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[2].

In many cultivars, blast-resistance is quite short-lived in field conditions as the pathogen mutates very often favored by the environment to spread the disease. Hence, breeding for more durably resistant cultivars has become a priority in rice improvement programmes throughout the world. Tremendous advancements in DNA marker technology together with the concept of marker-assisted-selection (MAS) might provide new solutions for selecting and maintaining more durable resistant genotypes in rice. Integrating molecular marker technologies such as MAS into breeding strategies could become increasingly important in the coming years, to realize the genetic gains with greater precision and accuracy.

Molecular markers are useful tools for monitoring gene introgressions and to detect polymorphism among species. The use of molecular markers can help in esti-

mating 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[3].

Several types of molecular markers are available today, including those based on restriction fragment length polymorphism (RFLP)[4], random amplified polymorphic DNA (RAPD)[5,6], amplified fragment length polymorphism (AFLP)[7], and simple-sequence repeats (SSRs) or microsatellite markers[8]. In RAPD technique, DNA polymorphisms are produced by "rearrangements or deletions at or between oligo-nucleotide primer binding sites in the genome"[5,6] as it provides a convenient and rapid assessment of the differences in the genetic composition of the related individuals and has been employed in a large number of plants for the determination and assessment of genetic diversity. They are dominant, small amounts of DNA required, quick and simple, inexpensive, multiple loci from a single primer is possible. Locus-specific, co-dominant PCR-based markers can be developed from RAPD markers. With the help of RAPD, genetic variations have been detected, both, within and between species of plants[9-14]. 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. (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.

EXPERIMENTAL

Plant materials

Twenty six cultivars of rice *Oryza sativa* L., representing 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, which includes 6 ARBN lines (Asian Rice Biotechnological Network) introgressed with leaf blast disease resistance genes.

Field screening for leaf blast disease reaction

All the rice genotypes were screened at Hybrid Rice Evaluation Centre, Gudalur, Tamil nadu, 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[15] on a 0-9 scale as detailed at 35th day after sowing, when the susceptible check (Bharti) was fully infected. 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, 1-2mm in breadth, more than 3mm long with brown margin. 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 %) percent was worked out using the formula given by[16] :

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

Artificial screening for leaf blast disease reaction

Artificial screening for rice blast disease was done at the Paddy breeding Station, Coimbatore, India, in a specially constructed screen house. It is provided with good irrigation facilities and it consisted of mist blowers, which can spray water in a fine mist inside the chamber. Subsequently, the seedlings were misted 4-5 times a day and the required temperature and humidity for disease manifestation was maintained throughout the observation period. The screen house was maintained at 32 to 37C (day temperature) and 94 to 96% relative

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TABLE 1 : Details of the rice genotypes used in this study and their geographic origin

S. No	Genotypes	Pedigree	Habit	Duration (days)	Place of collection	Geographic origin
1	Ajaya	IET 4141 / CR 987216	Semi dwarf	105	India	South Asia
2	ASD 16	ADT 39 / CO 39	Semi dwarf	110-115	India	South Asia
3	BPT 5204	GEB-24 / T(N) 1 / Mahsuri	Semi dwarf	140-145	India	South Asia
4	CB 98002	TNAU 89093 / ASD 5	Semi dwarf	130	India	South Asia
5	CB 98004	TNAU 89093 / ADT 40	Semi dwarf	130	India	South Asia
6	CB 98006	Ponni / CO 43	Semi dwarf	135	India	South Asia
7	CB 98013	CO 45 / IR 64	Semi dwarf	138	India	South Asia
8	Pusa Basmati	Pusa 167 / Karnal local	Semi dwarf	115	India	South Asia
9	IR 50	IR 2153-14 / IR 28 / IR 36	Dwarf	115	Philippines	South East Asia
10	ARBN 138	*Oryza minuta* (Acc. 10114) / (WHD-IS-1-127) / (DM 360)	Dwarf	135	Philippines	South East Asia
11	ARBN 142	BL 142	Semi dwarf	130	Philippines	South East Asia
12	IR 36	IR 1561-228 // IR 24 4 / *O. nivara* // CR 94-13.	Dwarf	110	Philippines	South East Asia
13	IR 64	IR 5657-3-3-3-1 / IR 2061-465-1	Semi dwarf	115-120	Philippines	South East Asia
14	Milyang 46	Doosan 8 / Sacheon 8	Dwarf	110	South Korea	South East Asia
15	Tadukan	Philippine *indica* cultivar (Luzon)	Semi dwarf	130-135	Philippines	South East Asia
16	Tetep	Vietnamese *indica* cultivar	Semi dwarf	130-135	Vietnam	South East Asia
17	TN 1	Chow-Woo-Gen / Tsai-Yuan-Chung.	Dwarf	120-125	Taiwan	South East Asia
18	White Ponni	Taichung 65/2 / Mayang Ebos- 80	Tall	125-130	Malaysia	South East Asia
19	ADT 43	IR 50 / Improved White ponni	Semi dwarf	110	India	South / S.E. Asia
20	CO 43	Dasal / IR 20	Dwarf	130-135	India	South / S.E. Asia
21	ARBN 153	C-101-Pai Kan Too (*japonica*)	Tall	110-115	China	Central Asia
22	ARBN 97	RIL 45 (Moroberekan / CO 39)	Semi dwarf	135	India	South Asia / Africa
23	ARBN 139	RIL 10 (Moroberekan / CO 39)	Dwarf	140	India	South Asia / Africa
24	ARBN 144	RIL 249 (Moroberekan / CO 39)	Semi dwarf	135	India	South Asia / Africa
25	Moroberekan	Guinean (West Africa) cultivar, *japonica*	Semi dwarf	130	Guinea (Africa)	Africa
26	Columbia - 2	Columbian *indica* cultivar	Semi dwarf	135	Columbia	Latin America

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 20 degrees Celsius. The varieties, TN 1, IR 50, TKM 9, CO 39 and ASD 16 were used as infector lines. The seeds of these varieties were mixed and were sown at the border rows of each bed. After a period of ten days, the entries to be screened were sown on the nursery beds in between infector rows. Each entry was sown in a single row with at least 25 seeds per row.

Artificial sporulation, inoculation and scoring

Severely blast affected leaves of TN 1, White Ponni, IR 50 and CO 39 showing large spindle-shaped lesions with dark-brown margin and greyish centre were collected as source for inoculation from the Paddy Breeding Station, Coimbatore during the wet season. Well-developed susceptible lesions were identified, excised and washed in running water for 15-20 min. The leaf bits were surface-sterilized with 0.01 per cent Mercuric Chloride solution for 45 seconds. They were then washed serially with sterile double-distilled water thrice and allowed for sporulation on sterilized glass slides by incubating them in a moist chamber at 28°C for 48 h. Well-sporulated lesions were placed in double-distilled sterile water in test tubes and vortexed for 1 min. About 1ml of spore suspension was added to sterilized plates and 4 per cent lukewarm agar was added. Single spores were located and picked up microscopically. Each spore was eventually transferred to potato dextrose agar (PDA) slants. The slants were incubated at 28°C for 2

days and stored at 4°C and the monosporic culture was used for inoculation. The same procedure described previously for artificial sporulation in their studies by[17] was followed.

The seedlings were sprayed with the spore suspension of the blast fungus cultured in the lab in oat meal agar medium, using a hand sprayer. The spore concentration was adjusted to 5×10^4 conidia/ml for inoculation[18]. Spraying of the blast spore suspension culture was done at regular intervals to enhance sufficient load of the fungus. The observation on the disease incidence was recorded; when the susceptible check (Taichung Native 1) was severely infected by blast. Individual plants in each entry were scored based on the leaf blast severity following Standard Evaluation System[15] on 0-9 scale at 25th day after sowing; the resistant check used was IR 64. Observations were recorded from 25 plants, when they were at third leaf stage. The disease reaction of each line was scored according to the Standard Evaluation System and classified.

DNA extraction

DNA from all the 26 genotypes were extracted and purified following the protocol described by[19] with slight modifications. DNA was checked for its purity and intactness and then quantified. The crude genomic DNA was run on a 0.8 percent agarose gel stained with Ethidium bromide following the protocol of[20] and was visualized in a gel documentation system (Alpha Imager ™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™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 mg per µl and stored at 4°C.

Molecular marker assay

Twenty six rice genotypes collected from different geographical regions 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. Out of 53 primers used to amplify twenty six rice genotypes, only 36 primers generated clear banding pattern. Amplification reactions were in volumes of 20µl containing 10mM Tris HCl (pH 9), 50mM KCl, 1.5mM MgCl2, 0.001 per cent gelatin, dATP, dCTP, dTTP and dGTP (each at 0.1mM), 0.2mM primer, 25-30mg 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 ™1200, Alpha Innotech Corp., California, USA).

Data analysis

Only the clear, unambiguous markers were scored. Each band was considered to be a single locus. Markers were scored for the presence and absence of the corresponding band among the genotypes. The scores '1' and '0' indicates the presence and absence of bands respectively. DNA band size was estimated by comparing DNA bands with a 1 Kb DNA ladder or lambda DNA *Eco* RI and *Hin*d III double digest (MBI Fermentas, India). To measure the informativeness of the markers, the polymorphism information content (PIC) was calculated. Marker index (MI) was calculated. Based on the MI, the primers were ranked and according to the rank first 5, 10 and 15 primers were selected and regarded as informative primers.

Genetic similarity (GS) between genotypes *i* and *j* was estimated by using Jaccard's coefficient, as described by[21]. Markers with missing observations for genotype *i* and/or *j* were not included in the calculation of GS*ij*. Based on the genetic similarity matrix, an unweighted pair group method of arithmetic average (UPGMA) cluster analysis was used to assess the pat-

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tern of diversity among the rice genotypes. All calculations were performed by using NTSYS-pc version 2.1 software[48]. Principal coordinate analysis (PCoA) was done to obtain a two dimensional scatter plot of individuals, in order to visualize the grouping between the individuals. The robustness of the clusters in the dendrogram was tested by bootstrapping with the software package WINBOOT, developed at the International Rice Research Institute[22].

RESULTS

Natural and artificial blast disease screening

Among the genotypes screened, highly significant lower mean disease reaction score (2.30 and 0.84) and mean PDI per cent (25.25 and 9.33) was recorded by Moroberekan in natural and artificial screening respectively. The higher mean disease reaction scores 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 total number of markers varied from 4 (OPM 17) to 17 (OPM 4 and OPBE 18) with a mean of 9.03 markers per primer. The number of polymorphic markers for each primer varied from 2 (OPE 18 and OPM 8) to 17 (OPM 4) with a mean of 6.80 polymorphic markers per primer (TABLE 4). The amplified product size ranged from 83bp to 2850bp. The PIC values ranged from 0.434 to 0.137, with a mean PIC value of 0.264. The marker index among the primers ranged from 7.378 to 0.332 with the mean marker index of 2.082.

Genetic diversity levels

Jaccard's coefficient of similarity ranged from 0.470 to 0.839 with a mean of 0.640. Most of the pair-wise similarity values fell into the range of 0.601-0.700. The genotypes Tadukan and ARBN 97 had highest 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. The average pair wise similarity values were calculated from the Jaccard's similarity coefficient values among the groups based on their geographical origin, among the South East Asian genotypes the value was 0.664, while it was 0.604 among the South Asian genotypes. The genotypes having the parentage of both South Asian/South East Asian origin had the average pair wise similarity values of 0.647, followed by the genotypes with parentage of South Asian/African origin with 0.646.

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, 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)**

Genetic diversity pattern

Cluster analysis was performed on the similarity in-

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	7.7.12	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, 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)**

dex calculated using by UPGMA (Un-weighted Pair-Group Method, arithmetic average) based on Jaccard's similarity coefficient and the cluster diagram was constructed.

The dendrogram revealed two major clusters, Cluster 1 and 2 which was further divided to five sub-clusters. 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

TABLE 4 : Details of amplicons produced by RAPD markers among rice genotypes

S. No.	Primer	Total no. of alleles	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**

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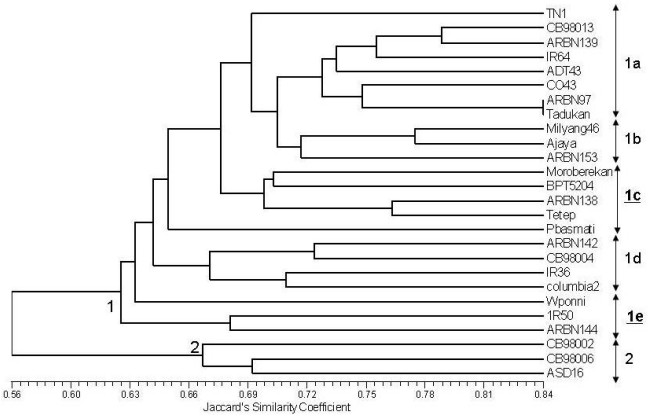

Figure 1 : Dendrogram of rice genotypes based on RAPD markers

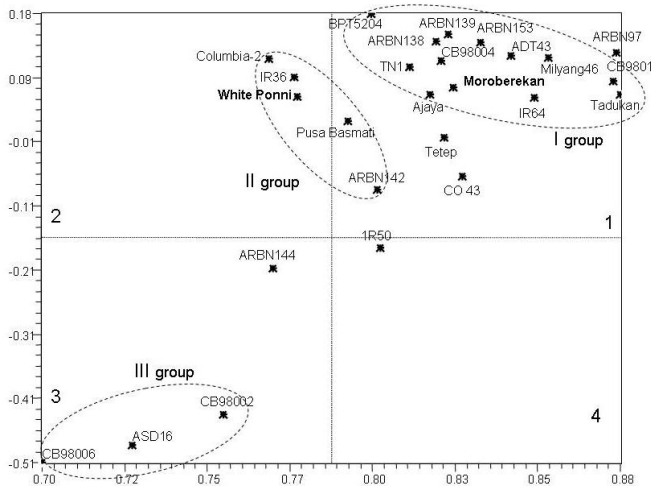

Figure 2 : Principal coordinate analysis of rice genotypes based on RAPD markers (*x* axis indicating the similarity coefficient and *y* axis indicating the genetic distances)

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) (Figure 1).

Principal co-ordinate analysis (PCoA)

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 2).

DISCUSSION

Efficient conservation of genetic diversity was not affected by the breeding system[23]. Since[24] first focused on the prospects of using wild relatives of crops as sources of genes for disease resistance, a great deal of effort has been expended in establishing germplasm collections of plant species. Extensive and well characterized collections of important species can provide unique raw materials for the production of new cultivars or act as the source of new traits that can be introduced into existing breeding materials. The genetic potential of major portion of the germplasm held in many gene banks is however, largely unknown, because it is poorly characterized. As a step towards the proper utilization and management of gene banks, a good genetic characterization of accessions is needed especially at the molecular level.

In the present study, the genetic diversity and the comparison of variability among the leaf blast resistant and susceptible genotypes was assessed. The germplasm materials used represent the collections from different geographical region. Eight genotypes were from South Asia, ten genotypes were from South East Asia, two from South Asia/South East Asia, one each from central Asia, Latin America and Africa and two genotypes were from South Asia/Africa (TABLE 1).

Fifty three random primers were used to amplify the DNA of the rice genotypes in this study. Thirty-six primers generated clear PCR amplified products. The number of primers used in this experiment was sufficient enough to characterize the genotypes, as previously the number of RAPD primers used by[25] was 22 RAPD primers to characterize 18 barley accessions, 18 primers to characterize 67 cocoa accessions[26], 36 primers for 40 genotypes of rice[11], 43 primers for 13 genotypes of rice[10], 10 primers for 18 genotypes of rice[27]. In the present experiment, Marker Index (MI) was calculated for multi-locus RAPD markers. MI reveals the amount of information that can be obtained

from a particular primer. Higher the MI, more the usefulness and informativeness of the primer. The marker index among the RAPD primers ranged from 0.336 to 7.378 in this analysis. The following five RAPD primers were with high marker index *viz.*, OPM 4 (7.378), OPC 6 (6.304), OPBE 18 (3.520), OPBE 3 (3.430) and OPM 5 (3.047) (TABLE 4). These primers might serve as informative and useful primers in future for large scale screening of germplasm for higher polymorphism.

PIC values are dependent on the genetic diversity of the genotypes chosen[28]. PIC provides an estimate of the discriminating power of the marker and it is a measure of allele diversity at a locus. The PIC value of RAPD markers can have 0.500 PIC value, since RAPDs are biallelic in nature. 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 0.434 to 0.137, which was in accordance to the results obtained by[29] with 0.0 to 0.500,[28] with 0.031 to 0.392.

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. Similar results were noticed by[30]. The results obtained were in accordance with the earlier findings that ancient cereal crop rice (*Oryza sativa* L.) was believed to be originated in India and adjoining South East Asia[31,32]. 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[32]. The view by[33] as China is one of the primary centres of origin cannot be ruled out because the number of genotypes representing the central Asian region was only one in this study.

A dendrogram was constructed based on Jaccard's similarity coefficient to infer relationship among the blast resistant and susceptible rice genotypes based on RAPD markers. It resulted in the discrimination of the genotypes into two major clusters and five sub clusters. The RAPD marker system was able to distinguish the genotypes based on their geographic origin with some exceptions.

Plants of a species growing in same environments and same ecological conditions for many years might adapt themselves to the prevailing conditions of that particular location and might tend to loss their divergence, in due course of time, were likely to be similar. Thus a species is likely to show a greater similarity in a region with similar climatic and ecological conditions. Further, the centres of origin of many species have shifted with time. The shift in diversity was brought about by a shift in the area of the greatest cultivation and due to the introduction of the species into an area with a greater ecological similarity than where the cultivar existed before.

The above concept cannot be ruled out in this study because the South East Asian varieties were either introduced into India more than 15-20 years back or after their introduction, they were improved by crossing with any of the South Asian cultivars *viz.*, TN 1 was introduced in India for its dwarfing gene (Dee-Gee-Woo-Gen) during the period of 1969. IR 64 was introduced in early 1990s and CO 43 was obtained by crossing IR 20 with Dasal during the year 1984. Hence, the South Asian and South East Asian genotypes were grouped in the same clusters right from sub cluster '1a' to '1e' was justified with obvious reasons, since the genotypes tend to loss their diversity in due course, as they got adapted to the South Asian regional and climatic conditions losing their divergence.

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[34] 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. Changes among individuals probably do not come from variations of a common ancestor, but from concurrent and independent

events, such as parallel development, co-evolution, specific adaptation, or others[35]. The above findings were congruent with the results obtained by[36].

Interestingly the Guinean cultivar, Moroberekan and the Latin American cultivar, Columbia-2 known for their durable blast resistance and cannot be singled out. They were grouped along with the South Asian and South East Asian cultivars, as Moroberekan was used extensively in blast and drought resistance breeding programmes by CRRI, Cuttack. There is no clear discrimination of the RAPD markers to distinguish leaf blast resistant and susceptible genotypes into separate clusters by the UPGMA clustering, since RAPD markers were not specifically designed for any particular trait.

The dendrogram constructed by RAPDs in the present study showed that both the South East and the South Asian accessions were more diverse and the accessions did not group into a single cluster as all the accessions were located in all the branches of dendrograms. This again confirms that South Asia (India) and South East Asia might be the primary centres of origin of rice. The ancient cereal crop rice (*Oryza sativa* L.) is believed to be originated in India and adjoining South East Asia[31, 32].

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[37]. Applying both methods was recommended to extract the maximum amount of information from the molecular (matrix) data[38]. Clustering was useful in detecting relationships among lines, while Principal Coordinate Analysis allowed a view on the relationships between groups.

Principal Coordinate Analysis was also done to show multiple dimensions of the distribution of the genotypes in a scatter-plot[39]. The scatter plot produced from principal coordinate analysis distributes the accessions along the two axes. Aggregation of individuals in a plot would reveal sets of genetically similar individuals. When the first three principal coordinates account for most of the variation (>35 per cent) of all the original variables, the scatter plot is considered to be the good representation of the data. Moreover, the relationships inferred were highly reliable.

In the present investigation, Principal Coordinate Analysis further validated the results of UPGMA cluster analysis. The first three principal coordinates based on RAPD markers, accumulated 98.06 per cent of total variation[40] reported that the first three principal coordinates accounted for 53.69 per cent of the genetic-similarity variance[29] reported first three principal coordinates accounted for 34% of the genetic-similarity variance. The first two principal coordinates explained 15.4% of the total variance[41,42] reported 39% variation for the first two coordinates[43] reported that first and second principal coordinates explained 52 and 7 per cent of variation, respectively[44] reported 70 per cent variation for the first three coordinates.

Scatter plot produced by the RAPD marker system distinguished the rice genotypes into three major groups belonging to South Asia (III group), South East Asia (II group) and the first principal coordinate (I group) does not discriminate any of the genotypes based on the geographical origin. In general, the principal coordinate analysis produced similar grouping pattern of genotypes as like the UPGMA analysis. 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[45]. 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[46]. 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 differ-

ent clusters based on the dendrogram. The genotype 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[47].

The selection of parents for the development of mapping population based on the genetic distance (similarity coefficient 0.627) between Moroberekan and White Ponni, and based on the dendrogram and the blast disease reaction scores was the very first report of its kind. The foremost criterion is the preference of the customers who chose white ponni as one of their favourite rice for cooking in southern parts of Tamil Nadu. Selection of parents for the development of mapping population also depends on the performance of parents based on the earlier reports.

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