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Full Length Research Paper

Predicting the relationship between molecular marker heterozygosity and hybrid performance using RAPD markers in rice (*Oryza sativa L.*)

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Successful prediction of heterosis and performance of F_1-hybrids from genetic similarity of their parents based on molecular markers has been reported in various crops. Estimation of genetic diversity and distance among various rice (*Oryza sativa* L.) genotypes and the correlation between genetic distance (GD) and hybrid performance would determine breeding strategies, define heterotic groups and predict future hybrid performance. In the present study, we surveyed genetic divergence among 26 rice genotypes using DNA markers and assessed the relationship between genetic distance and hybrid performance of 64 hybrids in a Line x Tester (4 lines x 16 testers) mating design. The 64 F_1 hybrids together with the 20 parents were evaluated for nine traits of agronomic importance, including yield, in a replicated field trial. The 20 parents along with the other six rice varieties were examined for DNA polymorphism using 53 random decamer oligonucleotide primers of which 36 primers generated clear banding profiles. A total of 245 polymorphic variants were generated and based on the polymorphism data, genetic distances (GDs) ranged from 0.23 to 0.53. Heterosis was observed in hybrids for most of the traits and yield exhibited the highest heterosis among the nine traits examined. The correlation values of GDs with F_1 performance were mostly non-significant, except for days to 50% flowering and test weight. The correlations of GDs with mid parent, better-parent heterosis and standard heterosis were not significant enough to be of predictive value. However, when specific combining ability (SCA) value was correlated with GD values of a group of hybrid (BPT 5204), there was high significance pertaining to a particular hybrid with relatively higher yield. These results indicated that GDs based on the random amplified polymorphic DNA (RAPD) markers may be useful for predicting heterotic combinations in rice and support the idea that the level of correlation between hybrid performance and genetic divergence is dependent on the germplasm used.

Key words: *Oryza sativa*, hybrid rice, predicting heterosis, random amplified polymorphic DNA, genetic diversity, genetic distance, specific combining ability, yield prediction.

INTRODUCTION

Rice (*Oryza sativa*) cultivated in Asia is one of the most diversified crop species, containing various ecological groups and ecotypes, with habitats ranging from tropical, subtropical to temperate zones. The gene pool of cultivated rice which consists of landraces and improved varieties belonging to different ecotypes provides genetic resources for hybrid rice breeding programmes. The identification of rice genotypes that form the superior hybrids is the most costly and time consuming phase in hybrid rice development. The prediction of hybrid performance in rice is of considerable importance and has

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attracted much interest. The yield potentials of rice cultivars have not been improved over the last two decades despite tremendous breeding efforts. This seems to suggest that the yield potentials of rice cultivars have reached a plateau. F_1 rice hybrids provide one approach to break the current yield ceiling (Kwon et al., 2002).

Predictors of single-cross hybrid yield value or heterosis between parental lines could therefore, increase the efficiency of hybrid breeding programs. The relationship between genetic distance (GD) and heterosis was reported before the development of genetic markers (Moll et al., 1965). With the development of molecular marker techniques, DNA polymorphisms have been used as markers to measure genetic diversity in many plant species. The relationship between heterozygosity and F_1 hybrid vigour remains unclear (Syed and Chen, 2005). For example, the association of GD with heterosis in elite inbred lines of corn may be very strong (Lee et al., 1989; Smith et al., 1990) or weak (Godshalk et al., 1990; Dudley et al., 1991), because the correlation between marker distance and F_1 performance depends on the origin of lines studied (Melchinger et al., 1990; Boppenmaier et al., 1993). In rice, there are also extensive studies on the relationship between molecular marker heterozygosity and hybrid performance, which also produced variable results. For example, Kwon et al. (2002) concluded that the correlation values of GDs with F_1 performance were mostly non-significant, except for yield, culm length and spikelets per panicle. The correlations of GDs with mid-parent and better-parent heterosis were not significant enough to be of predictive value which indicated that GDs based on the micro-satellite and RAPD markers may not be useful for predicting heterotic combinations in 'Tongil'-type rice in Korea. Sun et al. (2002) demonstrated highly significant positive correlations between heterosis of the F_1 yield and genetic distance of the parents, although the correlation between F_1 yield performance and genetic distance did not reach a significant level in rice. Zhang et al. (1995, 1996) evaluated the association of marker genotype heterozygosity with heterosis in a number of traits, including yield. Xiao et al. (1996) measured genetic diversity of 10 *japonica* and *indica* lines using RAPDs and SSRs and suggested that, although genetic divergence was useful for predicting yield potential and heterosis of intra-sub specific hybrids (*japonica*, *indica*), it was not useful in inter-sub specific hybrids (*indica* x *japonica*). Based on the result that the high correlation between the parental divergence and hybrid performance was largely due to high performance of inter-sub specific cross combinations, SaghaiMaroof et al. (1997) concluded that the level of correlation between marker distance and heterosis is dependent on the genetic materials employed. In the present study, the relationship between genetic distance and hybrid performance (relative heterosis, hetero-beltiosis and standard heterosis) along with specific combining ability values (SCA) was investigated in 4 groups of hybrid populations, with each group comprising of 16 hybrid combinations. This study was carried out using RAPD markers to assess the genetic diversity among 26 rice cultivars widely used in rice breeding programmes and to evaluate the association of the genetic diversity with F_1 performance and heterosis among 64 hybrids and 20 parents and to see whether diversity at the molecular level could be useful for predicting F_1 performance.

MATERIALS AND METHODS

Plant materials

Twenty six cultivars of rice (*O. 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 (Asian Rice Biotechnological Network) accessions introgressed with leaf blast disease resistance genes. Among the 26 rice cultivars, 20 cultivars were chosen for crossing programme. A non-reciprocal set of 64 crosses among these 20 cultivars was made in line x tester fashion [(4 lines (female) x 16 testers (male)] and the 64 F_1 hybrids along with the 20 parents were grown for phenotypic evaluation in a field at Paddy Breeding Station, Coimbatore. Field planting followed a randomized complete-block design with three replications. Each plot consisted of 40 plants planted in a single row with 15 cm spacing between plants and 25 cm spacing between rows. Phenotypic data were collected from the middle 20 plants randomly for nine traits as follows: (1) Days to 50% flowering, (2) plant height, (3) number of tillers per plant, (4) number of productive tillers per plant, (5) panicle length, (6) number of filled grains per panicle, (7) days to maturity, (8) 1000-grain and (9) grain yield per plant was measured as the weight of harvested grain per plant and recorded in grams. Means over replications were calculated for each trait and used in data analysis.

DNA extraction

DNA from all the 26 genotypes (Table 1) were extracted and purified following the protocol described by Gawal and Jarret (1991) with slight modifications.

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, Department 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 20 µl containing 10 mM Tris HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% 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

Table 1. Genotypes, pedigree, habit, duration, place of collection and their geographic origin of the rice genotypes used in this study.

S/N	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

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

and followed by 2 min at 92°C and 10 min at 72°C, respectively. Polymerase chain reaction (PCR) amplified products (15 µl) were subjected to electrophoresis in 1.5% agarose gels in 1 X TBE buffer at 60 V 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).

Statistical analysis

Only the clear, unambiguous markers were scored. 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).

The binary data matrices were entered into NTSYSpc package, Exeter Software, USA (Rohlf, 2000). The data were analyzed using qualitative routine to generate Jaccard's similarity coefficient. Similarity coefficients were used to construct dendrogram using UPGMA (unweighted pair group method with arithmetic average) and SHAN (sequential hierarchical and nested clustering) (Sokal and Michener, 1958). The principal coordinate analysis was constructed with NTSYS pc. To measure the informativeness of the markers, the polymorphism information content (PIC) and marker index (MI) for each locus was calculated. The PIC value provides an estimate of the discriminating power of the markers. Based on the MI, the primers were ranked and according to the rank, the first 5, 10 and 15 primers were selected and regarded as informative primers (data not shown). Genetic distance or general heterozygosity of an F_1 hybrid refers to the distance between the parents calculated using all the markers employed in a study (Zhang et al., 1994). Genetic distances (GDs) among all the 64 pairs of the 20 parents were estimated using 245 polymorphic bands according to the equation:

$$GD = 1 - 2N_{ij} / (N_i + N_j) \quad (1)$$

Where, N_{ij} is the number of bands common to lines *i* and *j* and (N_i + N_j) is the total number of fragments in both lines (Nei, 1973).

Heterosis was computed using three measurements: mid parent heterosis (calculated as the percentage of deviation from the mid-parent value), better-parent heterosis or heterobeltiosis (calculated as the percentage of deviation from the better-parent value) and the standard heterosis (calculated as the percentage of deviation from the standard check variety). The relationships between genetic distance and heterosis/hybrid performance were calculated by correlating simple correlation methods using heterosis or trait values and the SCA values on the genetic distances of the F_1 hybrids. The observations recorded on the hybrids and parents were subjected to line x tester analysis and the SCA effects of the crosses were worked out as suggested by Kempthrone (1957).

RESULTS

Level of polymorphism

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 (Table 2). 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 (Figure 1). The amplified product size ranged from 83 to 2850 bp. 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. Based on the higher marker index 5, 10 and 15 informative primer sets were identified (data not shown) and these primers produced a total of 72, 108 and 145 polymorphic markers, respectively.

Genetic similarity and genetic distance among the genotypes

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 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. The average pair wise similarity values were calculated from the Jaccard's similarity co-efficient values among the groups based on their geo-graphical 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 origins had the average pair wise similarity values of 0.647, followed by the genotypes with parentage of South Asian/African origin with 0.646.

The average genetic distance among the four groups of hybrids was 36.45% with a range from 23.00 to 52.97%. Most of the pair wise comparisons of genetic distances fell within the range of 30 to 40% (Table 3). BPT 5204 x CB 98006 was the most distant hybrid with a genetic distance of 52.97% followed by White ponni x CB 98002 (49.08%).

Cluster and principal coordinate analysis

The cluster analysis revealed two major clusters, Cluster 1 and Cluster 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 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

Table 2. Details of RAPD markers produced with blast resistant and susceptible rice genotypes

S/N	Primer	Total no. of bands	No. of polymorphic bands	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

PIC*, Polymorphic information content; MI#, marker index.

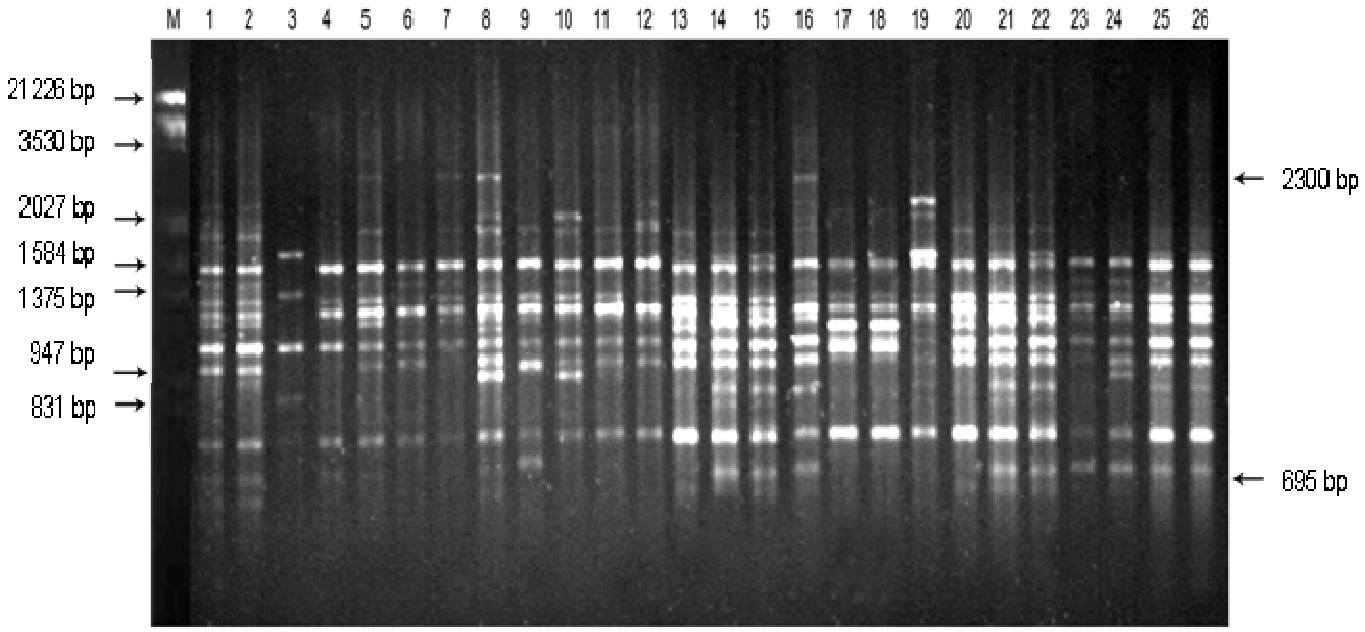

Figure 1. PCR electrophoretic profile of 26 rice genotypes (listed in Table 1 with same serial order from 1 to 26) M = λ (*Eco* RI + *Hin*d III) DNA marker.

Table 3. Genetic distances (complement of Jaccard's estimator) for the 64 rice hybrids calculated from RAPD marker data.

S/N	Hybrid combinations (Testers)	Genetic distance (%)			
		IR 50 (I line)	White ponni (II line)	TN 1 (III line)	BPT 5204 (IV line)
1	ARBN 97	30.20	35.40	30.60	31.00
2	ARBN 138	41.50	33.30	35.10	28.80
3	ARBN 139	37.30	38.70	30.40	35.20
4	ARBN 142	40.00	43.70	32.60	40.08
5	ARBN 144	32.25	45.73	40.10	43.60
6	ARBN 153	41.63	35.32	34.00	34.00
7	IR 64	32.30	39.70	27.60	36.20
8	CB 98002	33.83	49.08	45.90	42.70
9	CB 98004	39.66	38.80	35.00	37.65
10	CB 98006	41.46	41.60	48.10	52.97
11	CB 98013	29.90	37.20	23.00	34.40
12	Columbia 2	43.34	43.80	40.40	39.92
13	Milyang - 46	30.70	34.62	28.80	30.74
14	Moroberekan	38.80	37.35	33.30	30.04
15	Tadukkan	27.00	33.10	30.70	30.70
16	Tetep	36.24	34.10	40.20	31.38
i	Mean	36.00	38.84	34.74	36.21
ii	Minimum	27.00	33.10	23.00	28.80
iii	Maximum	43.34	49.08	48.10	52.97
iv	Std. Deviation	5.15	4.77	6.72	6.44
v.	Std. Error	1.29	1.19	1.68	1.61

Total number of groups (4), total number of crosses (64), overall mean of GD for all crosses = 36.45, mean standard deviation for overall crosses (SD) = 5.88 and mean standard error for all crosses (SE) = 0.74.

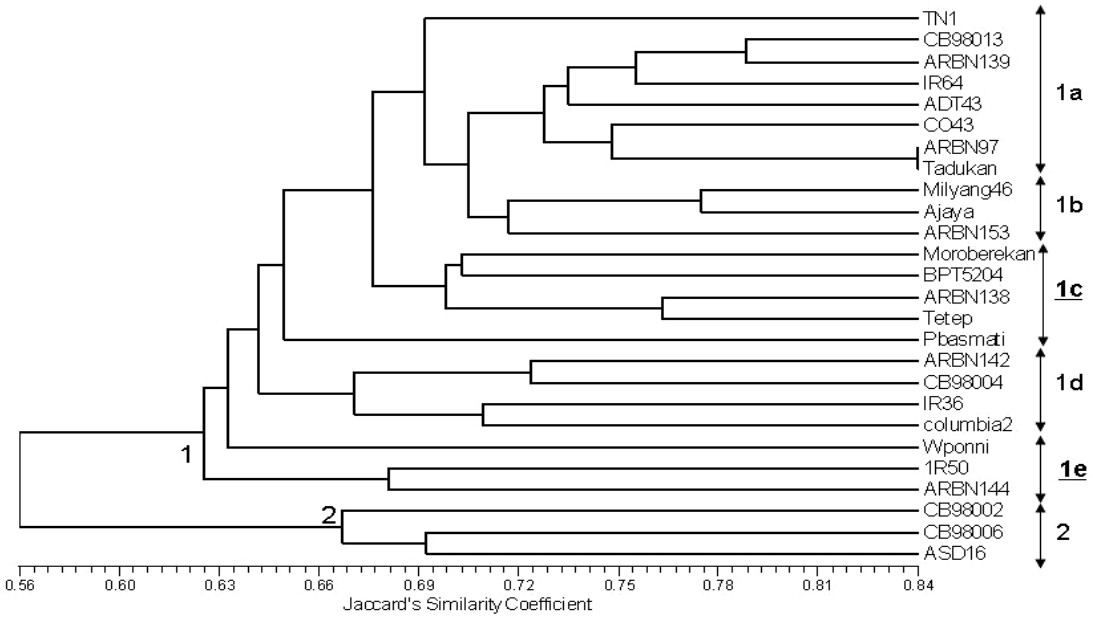

Figure 2. Dendrogram of 26 rice genotypes based on RAPD analysis of 36 RAPD primers using the Jaccard's similarity coefficient and UPGMA clustering.

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 2).

Three major groups of accessions were distinguished by scatter plot (Figure 3). The principal coordinate 1, 2 and 3 encompassed 89.27, 6.07 and 2.72% of total variation, respectively. The PcoA discriminated the genotypes into various groups based on the geographic origin comparatively in a better way than the UPGMA clustering.

Hybrid performance, heterosis and their correlations with genetic distances

The mean values, ranges of performance and heterosis of the 64 F_1 hybrids are given in Table 4. The degree of heterosis showed variation from trait to trait. For heterobeltiosis (heterosis over the better parent), number of tillers/plant showed the highest heterosis (50.62%), followed by number of productive tillers/plant (42.88%), 1000 grain weight (21.59%) and grain yield/plant (11.40%). Days to flowering (-9.99%) and days to maturity (-7.02%) exhibited significant negative heterosis. However, better-parent heterosis of filled grains/panicle was not statistically significant although it ranged from -38 to 48%, depending on the crosses. Mid-parent heterosis for yield varied from -31.9 to 71.9% in the hybrids. Better-parent heterosis for yield ranged between -42.31 and 78.9%. Days to 50% flowering, when compared with other traits, exhibited a low level of heterosis. Standard heterosis was obtained by using the standard check (ASD 16) as control, which is a variety for grain yield in Tamilnadu, India. Standard heterosis for yield varied from -42.3 to 80.3% in the hybrids.

Simple correlation coefficients between the GD of the parents and mean values of heterosis (relative heterosis) (di), mid parent heterosis (dii) and standard heterosis (diii) in yield and its components and SCA values were calculated in the entire set of 64 crosses (Table 5). This shows that correlation coefficients between the GD of parents and heterosis and SCA values have not reached significant levels, when the overall values were considered. When the individual groups of hybrids were considered, the group I hybrids, relative and standard heterosis was significantly and positively correlated with genetic distance for days to fifty per cent flowering (r = 0.485* and r = 0.588*, respectively). Test weight was significantly and positively correlated for relative heterosis and heterobeltosis with genetic distance with r = 0.487* and r = 0.511*, respectively. Among the group II hybrids, number of tillers per plant was significantly and positively correlated for heterobeltosis (dii) with genetic distance with r = 0.489*. Among the group III hybrids, relative heterosis was negatively significantly correlated with genetic distance for number of tillers per plant and filled grains per panicle with r = -0.558* and r = -0.496*, respectively. The heterobeltiosis for the traits, number of tillers per plant, number of productive tillers per plant and filled grains per panicle were negatively significantly correlated with GD (r = -0.507*, r = -0.482*, respectively). Among the group IV hybrids, where BPT 5204 was used as line, there was no significant positive correlation for any of the characters studied, but all three heterotic values for grain yield per plant were found to be positive

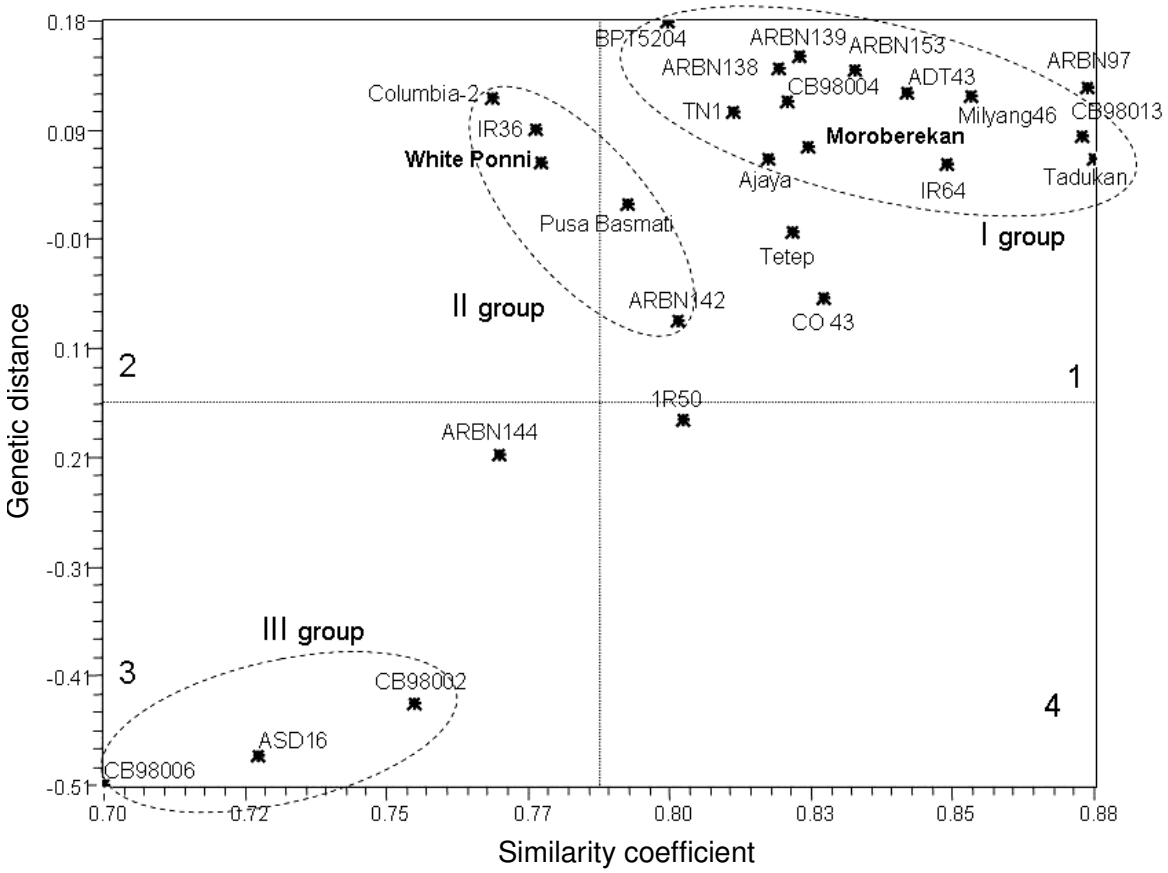

Figure 3. Principal coordinate analysis of rice genotypes based on RAPD markers.

and relatively close to significance at 1% level. The pooled heterosis values of relative, mid parent and standard of all the 64 hybrids was not significant enough to predict the hybrid performance in any of the traits studied.

SCA and their correlations with genetic distances

Burstin et al. (1994) found that relative proportion of SCA effect was an indicator of the degree of hybrid performance prediction by genetic distance of parental inbreds. Specific combining ability (SCA) effects of grain yield and yield attributes were correlated with genetic distances among the four groups of hybrids and all of the groups pooled together (Table 5). Among the group I hybrids, SCA was positively significantly correlated with genetic distance for plant height (r = 0.492). Among the group III hybrids, the SCA effects of days to fifty per cent flowering and number of productive tillers per plant was negatively significantly correlated with genetic distance (r = -0.515* and r = -0.486*, respectively). Among the group IV hybrids, SCA effects for grain yield was positively and highly significantly correlated with genetic distance (r = 0.609**) (Figure 4).

DISCUSSION

In the present study, fifty three random primers were used to amplify the DNA of the rice genotypes. Thirty-six primers generated clear PCR amplified products. The number of primers used in this experiment was sufficient enough to characterize the genotypes. Previously, the number of RAPD primers used by Russell et al. (1997) was 22 RAPD primers to characterize 18 barley accessions, 18 primers to characterize 67 cocoa accessions (Lerceteau et al., 1997), 36 primers for 40 genotypes of rice (Ravi et al., 2003), 43 primers for 13 genotypes of rice (Kwon et al., 2002) and 10 primers for 18 genotypes of rice (Raghunathachari et al., 2000). In the present investigation, RAPD markers produced 75.38% polymorphism in average with 245 polymorphic markers out of 325 amplicons. Similar results were obtained (Yu and Nguyen, 1994; Virk et al., 1995; Mezeneer et al., 1997; Sonnante et al., 1997; Khandelwal et al., 2005) in rice using RAPD markers. Marker index (MI) was calculated for multi-locus RAPD markers. MI reveals the amount of information that can be obtained from a particular primer. The higher the MI, the more the usefulness and informative the primer will be. In the present study, the marker index among the RAPD primers ranged from 0.336 to 7.378.

Table 4. Mean values, ranges of performance and heterosis among the 64 F_1 hybrids.

Trait	Performance		MP heterosis (%)[1]		BP heterosis (%)[2]		Std. heterosis[3]	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Days to 50 % flowering	97.39**	76.4 to 119.8	-2.6**	-17 to 14	-9.99**	-27.1 to 1.7	23.60**	-3.0 to 52.3
Plant height	95.90**	73.4 to 140.6	17.0**	4.5 to 50	9.14**	-19.8 to 42.0	21.40**	-7.0 to 78.0
No. of tillers/plant	31.16**	17.0 to 56.0	67.7**	-22.0 to 198	50.62**	-31.8 to 184	73.09**	-5.56 to 211
No.of productive tillers/ plant	25.58**	14.4 to 42.6	57.2**	-18.5 to 167	42.88**	-21.0 to 160	68.28**	-5.26 to 180
Panicle length	23.21**	17.6 to 27.7	1.55	-22.9 to 18.7	2.56*	-28.0 to 15.4	3.15**	-22.1 to 22.5
No.of Filled grains/panicle	126.10**	94.0 to 162.4	9.15**	-30.1 to 52.2	1.16	-38.01 to 48.0	14.00**	-15.0 to 46.8
Days to maturity	127.56**	96.0 to 146.0	1.65*	-25.7 to 14.7	-7.02**	-30.0 to 9.26	16.81**	-11.9 to 34.2
1000 grain weight	19.76**	15.7 to 23.8	18.30*	-21.3 to 19.0	21.59**	-24.24 to 16.2	21.68**	-21.0 to 22.4
Grain yield/plant	27.97**	15.37 to 46.40	9.26**	-31.6 to 71.9	11.40**	-42.31 to 78.9	12.76**	-42.3 to 80.3

*, ** Significant at P = 0.05 and P = 0.01, respectively; 1 mid-parent heterosis; 2, better-parent heterosis; 3, standard heterosis

The following five RAPD primers 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) 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 (Manimekalai and Nagarajan, 2006). 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, 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 Hongtrakul et al. (1997) with 0.0 to 0.500, Manimekalai and Nagarajan (2006) with 0.031 to 0.392 and Subudhi et al. (2005) with 0.150 to 0.334. The MI and PIC values give an idea about the discriminative power of markers.

Jaccard's (1908) coefficient of similarity was employed in this investigation, after considering the type of markers (dominant markers) resulting from RAPD analysis. The similarity coefficient reveals the level of similarity among the genotypes. In the present study, the mean similarity co-efficient value obtained was 0.640. The lowest genetic similarity was obtained between BPT 5204 and CB 98006 (0.470). The highest value was obtained between ARBN 97 and Tadukkan (0.839). Wider variation of similarity was observed for the RAPDs and it might be due to sharing completely different alleles among the genotypes which reveals the better discriminating power of the RAPD markers. In the present investigation, Jaccard's similarity based on RAPD skewed towards higher values. The superiority of RAPD based polymor-phism detection has been reported earlier by Chowdari et al. (1998) with values ranging from 0.50 to 0.81, 0.350 to 0.870 (Paul et al., 1997) and 0.280 to 0.930 (Lu et al., 2002).

A dendrogram was constructed based on Jaccard's similarity coefficient to infer relationship among the rice genotypes based on RAPD markers. It resulted in the discrimination of the genotypes into two major clusters and six sub clusters. The RAPD marker system was able to distinguish the genotypes based on theirgeographical origin with some exceptions. The cluster '1a' consisted of eight genotypes, in which four of which belong to South East Asian geographical origin (TN 1, ADT 43, IR 64 and Tadukan), each one from South East/ South Asia (CO 43), South Asia (CB 98013) and two genotypes (ARBN 97, ARBN 139) from South Asia/Africa. All these genotypes were cultivated in India except ARBN 97 and ARBN 139. The genotypes ARBN 97 and ARBN 139 had CO 39 as the common parent in their pedigree records, which belongs to South Asian origin and this might be the reason for the two genotypes clustering together. The dendrogram constructed by RAPDs in the study showed that both the South East and the South Asian acces-sions 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 (*O. sativa* L.) is believed to have originated in India and adjoining

Table 5. Simple correlations of general heterozygosity (Genetic Distance) based on the RAPD marker data with mean values of heterosis and specific combining ability effects (SCA) for yield and it's attributes among four hybrid groups.

Character	Mean heterosis/SCA	Group I(IR 50)	Group II white ponni)	Group III (TNI)	Group IV (BPT 5204)	Pooled group values
Days to 50 % flowering	(di)	0.485*	0.036	-0.310	-0.363	-0.005
	(dii)	0.275	0.327	-0.156	-0.190	0.071
	(diii)	0.588*	0.328	-0.317	-0.193	0.106
	SCA	0.245	0.318	-0.515*	-0.252	-0.081
Plant height	(di)	0.130	0.026	-0.362	-0.364	-0.08
	(dii)	0.108	0.016	-0.224	-0.412	-0.161
	(diii)	0.139	0.017	-0.226	-0.315	0.061
	SCA	0.492*	0.160	-0.327	-0.527*	-0.123
Number of tillers per plant	(di)	-0.514*	0.372	-0.558*	-0.193	-0.241*
	(dii)	-0.535*	0.489*	-0.507*	0.110	-0.232*
	(diii)	-0.555*	0.232	-0.555*	-0.081	-0.282*
	SCA	-0.423	0.308	-0.453	-0.353	-0.083
No. of productive tillers/plant	(di)	-0.413	0.213	-0.365	0.330	-0.078
	(dii)	-0.513*	0.356	-0.507*	0.184	-0.181
	(diii)	-0.531*	0.387	-0.485*	0.183	-0.189
	SCA	-0.579*	0.292	-0.486*	0.141	-0.201
Panicle length	(di)	0.248	-0.097	-0.291	-0.095	0.009
	(dii)	0.109	-0.135	-0.259	0.177	-0.019
	(diii)	0.133	0.255	-0.258	0.088	0.033
	SCA	0.233	0.125	-0.219	0.019	-0.005
Filled grains per panicle	(di)	-0.063	-0.287	-0.496*	0.358	-0.166
	(dii)	0.022	-0.103	-0.482*	0.352	-0.088
	(diii)	0.052	-0.204	-0.447	0.365	-0.121
	SCA	0.209	-0.177	-0.434	0.503	0.056
Days to maturity	(di)	0.392	-0.019	0.295	-0.144	-0.078
	(dii)	0.256	0.084	0.254	-0.057	-0.11
	(diii)	0.378	0.065	0.294	-0.058	0.039
	SCA	0.135	-0.059	0.347	0.167	-0.155
Test weight	(di)	0.487*	-0.484*	-0.122	-0.204	-0.063
	(dii)	0.511*	-0.486*	-0.062	-0.208	-0.041
	(diii)	0.286	0.329	-0.141	-0.087	-0.062
	SCA	0.248	0.281	-0.019	0.039	0.080
Grain yield per plant	(di)	-0.345	-0.389	-0.354	0.410	-0.125
	(dii)	-0.328	-0.247	-0.405	0.404	-0.090
	(diii)	-0.329	-0.225	-0.406	0.366	-0.122
	SCA	-0.049	-0.038	-0.212	0.609**	-0.134

*, **Significant at 5 and 1% levels, respectively; di, relative heterosis; dii, heterobeltosis; diii, standard heterosis; SCA, specific combining ability.

South East Asia (Chang, 1985; Sarla et al., 2005).

The extensively used hierarchical methods, such as UPGMA, might not be appropriate for the clustering of genotypes if the materials studied were intra-specific in nature. Hence, principal coordinate analysis might be appropriate (Chaparro et al., 2004). 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 co-ordinates account for most of the variation (> 35%) 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 this study, 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 geo-graphical origin.

RAPD markers were proposed as an approach to assess genetic divergence among genotypes (Santos et al., 1994). Genetic distances were calculated for 64 F_1 hybrids between pairs of lines on the basis of the method developed by Nei and Lei (1979) from the Jaccard's similarity coefficient matrix for the rice genotypes. Genetic distance was estimated for the four groups of hybrids *viz.,* Group I (IR 50), Group II (White ponni), Group III (TN 1) and Group IV (BPT 5204). Each group comprised of a

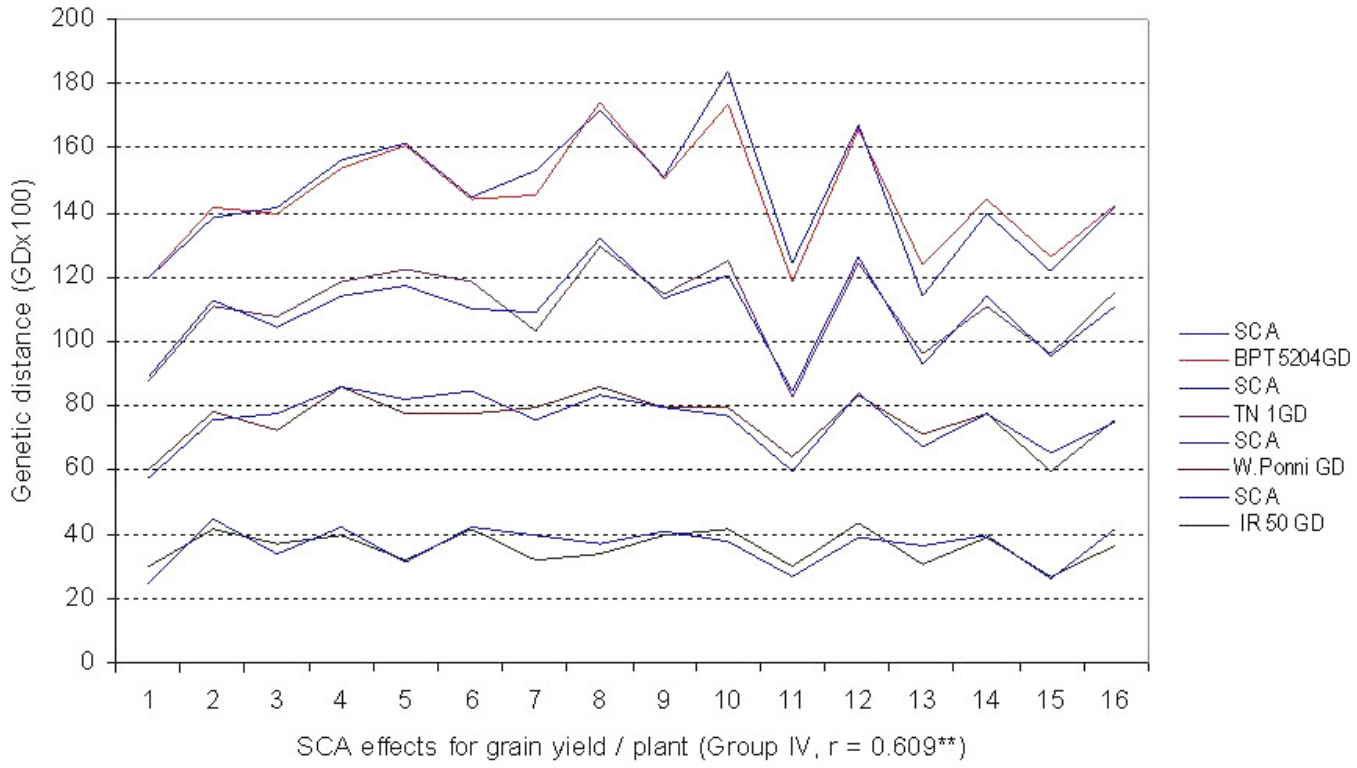

Figure 4. Association of genetic distances (GD) versus SCA effects grain yield per plant in 64 hybrids.

line and 16 testers.Among the Group I (IR 50) hybrids, when the standard heterosis values were correlated with the genetic distance values, relative and standard heterosis was significantly and positively correlated with genetic distance for days to fifty per centflowering with r = 0.485* and r = 0.588*, respectively. The first five top ranking hybrids (data not shown) with earlier flowering based on the standard heterosis and the mean value were comprised by the group I hybrids *viz.,* IR 50 X IR 64, IR 50 X CB98013, IR 50 X Milyang 46, IR 50 X ARBN 142 and IR 50 X ARBN 144. Hence, from the present study, it is evident that the RAPD markers were able to predict the hybrid group (Group I) with earlier flowering type. Test weight was significantly and positively correlated for relative heterosis and heterobeltiosis with genetic distance of r = 0.487* and r = 0.511*, respectively, among the Group I hybrids. When the result was compared with the top five ranking hybrids based on the mean value and the standard heterosis value, the hybrids IR 50 X IR 64 and IR 50 X ARBN 153 ranked first and fourth, respectively, which confirms the prediction of the trait by RAPD markers. Apart from the Group I hybrids there is no clear cut prediction or correlation of the heterosis value with the genetic distance for any of the traits studied except in the Group IV (BPT 5204) hybrids, all three heterotic values (di, dii and diii) for grain yield per plant were found to be positive and relatively close to significance at 1% level. Similar results were obtained by Betran et al. (2003) where there is a positive correlation between the grain yield and the genetic distance but the values were not significant. The pooled heterosis values of relative, mid parent and standard of all the 64 hybrids was not significant enough and very weak to predict the hybrid performance in any of the traits studied. Since the degree of heterosis depends on the relative performance of the parents and the corresponding hybrids. Environment can differentially affect the performance of the parental lines and hybrids, altering the relationship between the genetic distance and the heterosis (Betran et al., 2003). Similar results in which the genetic distance was not correlated with the hybrid performance of crosses was noticed (Godshalk et al., 1990; Boppenmair et al., 1990; Melchinger et al., 1990; et al., 1992; Marsan et al., 1998). Zhang et al. (1994) concluded that the distance based on the random genetic markers is a poor predictor of heterosis, possibly due to the noise resulting from markers not linked directly to the trait being studied (Bernardo, 1992).

In the present study, the SCA effects for grain yield in the Group IV hybrids were positively and highly significantly correlated with genetic distance (r = 0.609**). This result was further confirmed by the values obtained among the top five ranking hybrids selected based on the higher SCA values for grain yield per plant. The hybrids BPT 5204 X CB 98006 (10.57**) and BPT 5204 X IR 64 (7.94**) ranked first and second, respectively, among the

superior grain yielding varieties.

The poor association between PCR marker-based diversity and heterosis found in this study might require consideration of the usefulness of DNA markers for selecting hybrid combinations rice cultivars and require testing of more genotypes in hybrid combinations. As more PCR-based markers, especially SSRs which may provide a more detailed coverage throughout the rice genome are developed, a significant improvement in identifying tight associations between markers and quantitative trait loci (QTL) would be possible (Temnykh et al., 2000, 2001). The use of selected DNA markers linked to QTLs for yield and other yield-related traits may also lead to a better correlation between hybrid performance and genetic divergence, as suggested by Zhang et al. (1996).

In summary, these results indicate that GDs based on the microsatellite and RAPD markers may be useful for predicting heterotic combinations in the rice genotypes selected and are supportive of the idea that the level of correlations between hybrid performance and genetic divergence is dependent on the germplasm employed.

Abbreviations

GD, Genetic distance; **RAPD,** random amplified polymorphic DNA; **SSRs,** simple sequence repeats, **SCA,** specific combining ability; **CRRI,** Central Rice Research Institute; **ARBN,** Asian rice biotechnological network, **UPGMA;** unweighted pair group method with arithmetic average, **SHAN;** sequential hierarchical and nested clustering, **PIC;** polymorphism information content, **MI;** marker index.

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