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Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers

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Abstract Ten elite inbred lines (four *japonica*, six *indica*), chosen from those widely used in the hybrid rice breeding program at Human Hybrid Rice Research Center in China, were crossed to produce all possible hybrids excluding reciprocals. The 45 F_1 hybrids along with the ten parents were evaluated for eight traits of agronomic importance, including yield potential, in a replicated field trial. The ten parents were analyzed with 100 arbitrary decamer oligonucleotide primers and 22 microsatellite (simple sequence repeats, SSRs) primer sets via polymerase chain reaction (PCR). Out of the 100 random primers used, 74 were informative and amplified 202 non-redundant bands (variants) with a mean of 2.73 bands per polymorphic primer. All 22 microsatellite primer sets representing 23 loci in the rice genome showed polymorphisms among the ten parents and revealed 90 alleles with an average of 3.91 per SSR locus. Cluster analysis based on Nei's genetic distance calculated from the 291 (202 RAPDs, 89 SSRs) non-redundant variants separated the ten parental lines into two major groups that corresponds to *indica* and *japonica* subspecies, which is consistent with the pedigree information. Strong heterosis was observed in hybrids for most of the traits examined. For the 43 diallel crosses (excluding 2 crosses not heading), yield potential, its components (including panicles per plant, spikelets per panicle and 1000-grain weight) and their heterosis in F_1 hybrids showed a significant positive correlation with genetic distance. When separate analyses were performed for the three subsets, yield potential and its heterosis showed significant positive correlations with genetic distance for the 15 *indica* × *indica* crosses and the 6 *japonica* × *japonica* crosses; however, yield potential and its heterosis were not correlated with genetic distance for the 22 *indica* × *japonica* crosses. Results indicated that genetic distance measures based on RAPDs and SSRs may be useful for predicting yield potential and heterosis of intra-subspecific hybrids, but not inter-subspecies hybrids.

Key words Rice · Heterosis · RAPDs · Microsatellites · Genetic distance

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Introduction

Prediction of heterosis is interesting to breeders of crops like rice and maize in which hybrids are commercially important. Screening combinations for superior F_1 performance and strong heterosis is the most costly and time-consuming process in hybrid rice breeding programs. It is estimated that at least 20,000 rice crosses are made and evaluated in fields each year at Hunan Hybrid Rice Research Center, China, to identify combinations possessing high yielding potential (L. P. Yuan, personal communication). Those hybrids possessing high yielding potential become targets for the transfer of key genes (genes for fertility restoration, cytoplasmic male sterility, photosensitive or thermosensitive male sterility and wide compatibility) absolutely necessary for the exploitation of heterosis in rice (Yuan 1987). If a simple, efficient, inexpensive and reliable method could be used to predict heterosis prior to expensive field testing, much of the field work associated with making crosses and field evaluation would be eliminated and hybrid rice breeding programs would be accelerated.

The level of genetic diversity between two parents has been proposed as a possible predictor of F_1 performance and heterosis in rice. This proposition stems from studies by Lin and Yuan (1980), Yuan and Cheng (1986)

and Yuan (1985). They concluded that hybrids showing strong heterosis were usually developed from parental lines diverse in relatedness, ecotype, geographic origin, etc.

Attempts have been made to relate isozyme diversity to F_1 performance and heterosis in rice in several instances. Deng and Wang (1984) examined the isozyme spectra and heterosis of 160 F_1 hybrids and concluded that four patterns of isozyme spectra (complementary, dominant, heterozygous and distinctive) were associated with heterosis, with the complementary pattern being the most important. Li et al. (1982) demonstrated that dominant complementary bands in the esterase iso-zymes of hybrids were correlated with the magnitude of heterosis. However, Peng et al. (1988) found no correlation between heterosis and isozyme variation in the 75 F_1 hybrids created from 18 parental lines.

DNA polymorphisms have been extensively employed as a means of assessing genetic diversity. Restriction fragment length polymorphisms (RFLPs) have been utilized to study genetic diversity and its relationship to heterosis in maize (Boppenmaier et al. 1992; Dudley et al. 1991; Godshalk et al. 1990; Lee et al. 1989; Melchinger et al. 1990a, b, 1992), rice (Xie 1993; Zhang et al. 1994, 1995) and oats (Moser and Lee 1994). The conclusions from these studies regarding the use of RFLPs for predicting heterosis were variable.

The polymerase chain reaction (PCR) provides a simpler, faster, safer and less expensive means for genome analysis compared with RFLPs. A single, short oligonucleotide primer can amplify specific sequences of genomic DNA through PCR. Randomly amplified polymorphic DNA sequences (RAPDs) obtained by the use of random oligonucleotide primers in PCR have been extensively used as molecular markers for tagging genes (Borokova et al. 1995; Chunwongse et al. 1994; Martin et al. 1991; Ronald et al. 1992), saturating existing molecular maps (Giovannoni et al. 1991), constructing molecular maps (AI-Janabi et al. 1993; Lefebvre et al. 1995, Link et al. 1995). Recently, simple sequence repeats (SSRs), termed microsatellites, have been used as genetic markers in many species (e.g. human, mouse, pig, rice, barley, maize, arabidopsis, grape, soybean) because the difference in the number of repeats can be detected via PCR using SSR primers. Wu and Tanksley (1993) demonstrated in rice that microsatellites show much more polymorphism than RFLP markers. This suggests that such markers would be ideally suited to studying genetic diversity in rice.

In the study presented here, RAPD and microsatellite markers were employed to study the genetic diversity among ten elite parental lines widely used in hybrid rice breeding programs in China. The relationship of genetic diversity with hybrid performance and heterosis was examined to assess whether such PCR-based markers were useful for evaluating germplasm and predicting F_1 performance and heterosis in rice.

Materials and methods

Parental lines and crosses

Ten elite parents were selected from those commonly used in hybrid rice breeding programs at Hunan Hybrid Rice Research Center, China. These lines fall into two groups based on their morphological characteristics: the *indica* group includes 'Xin Te Qing' (hereafter called XQ), 'CT 203' (CT), 'Min Hui 63' (MH), 'Ce 49' (CE), 'IR 56' (IR) and 'R402' (R4); and the *japonica* group includes 'Nipponbare' (NP), 'Ji Leng 89-199-1' 'Chang Bai 7' (CB) and 'Rong Guang 66' (RG).

A diallel set of 45 crosses (excluding reciprocals) was made during the summer of 1993 at the Hunan Hybrid Rice Research Center.

Field evaluation and data collection

The 45 F_1 hybrids along with the ten parental lines were grown for phenotypic evaluation in a field in a randomized complete block design with two replications (plots) in the summer of 1994 at the Hunan Hybrid Rice Research Center, China. Thirty-three plants (three lines × 11 plants per line) were planted at a density of 300,000 plants per hectare in each of 110 plots. The middle 5 plants in the central line of each plot were used for data collection.

Eight agronomically important traits were examined: days to heading, days to maturity, plant height (centimeters), panicle length (centimeters), panicles per plant, spikelets per panicle, 1,000-grain weight (grams) and spikelets per plant. Means over replications were calculated for each trait and used in data analysis.

Partial-sterility is a common phenomenon in F_1 hybrids between *indica* and *japonica*, the two subspecies of *Oryza sativa* (Jennings 1966; Oka 1974, 1988; Xiao 1989; Xiao and Yuan 1988, Yuan et al. 1989). Partial-sterility was also observed in the current study with an average of 42.63% seed set over the 22 *indica* × *japonica* hybrids. Therefore, grain yield was not measured. Instead, yield potential was calculated based on its components. As yield potential is a function of its components, yield potential (grams/plant) = 1/1000 × number of panicles per plant × spikelets per panicles × 1,000-grain weight × 75% (normal seed set rate – portion of spikelets which are able to produce seeds). Yield potential is the most important factor in deciding whether a hybrid combination is going to be targeted to receive the wide compatibility genes which confer F_1 fertility (75% or more seed set) (Ikehashi and Araki 1985) in *indica* × *japonica* hybrid rice breeding programs (Yuan 1987).

RAPD amplification and assay

The set of 100 arbitrary decamer oligonucleotide primers used in this study has been previously shown to amplify rice genomic DNA (Xiao and Tanksley, unpublished results). Amplification reactions were in a final volume of 25 µl containing 10 ng of genomic DNA, 0.2 µ*M* primer, 200 µ*M* each of dATP, dTTP, dGTP and dCTP, 0.5 unit of *Taq* DNA polymerase, 10 m*M* TRIS-HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin. Samples were covered with mineral oil and amplified through 45 cycles of 1 min at 94 °C, 1 min at 35 °C, 2 min at 72 °C, followed by a final extension at 72 °C for 7 min in a PTC-100™ Programmable Thermal Controller (MJ Research). Amplified products were resolved by electrophoresis in 2% agarose gels.

Microsatellite amplification and assay

In addition to the 100 random primers, 22 microsatellites were used to amplify short sequence repeats (SSRs) of genomic DNA from the ten parents. Of the 22 primer sets, 21 showed single locus amplification of short sequence repeats, 1 amplified 2 loci. All 23 loci have been previously placed on a rice molecular map: RM1 through RM14, RM16, RM18, RM19, RM20A, RM20B, RM21 and RM22 (Panaud et al. 1996); and RM122 and RM164 (Wu and Tanksley 1993). The polymerase chain reactions were performed in a volume of 50 µl containing 20 ng of genomic DNA, 0.2 µ*M* of each primer, 200 µ*M*

each of dATP, dTTP, dGTP and dCTP, 1.0 unit of *Taq* DNA polymerase, 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin. Reactions were covered with mineral oil and processed in a PTC-100™ Programmable Thermal Controller (MJ Research) programmed for 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C, with a final extension at 72 °C for 5 min. After amplification, 25 µl of stop solution was added to the PCR products, and they were denatured at 94 °C for 2 min. Five microliters of each reaction were run on a 4% polyacrylamide denaturing gel containing 7 *M* urea.

A silver-staining procedure was employed to reveal bands after electrophoresis as described in Cho et al. (1996). The plate on which the gel was bound was soaked and gently shaken in a 10% acetic acid solution for 20 min. After three 2-min washes with ddH_2O, the plate was incubated in staining solution containing 0.2% (w/v) silver nitrate and 0.05% formaldehyde for 30 min. It was then immersed in ddH_2O for 10 s and transferred to cold (10 °C) developing solution containing 3% (w/v) sodium carbonate, 0.0002% sodium thiosulfate (w/v) and 0.05% formaldehyde. An equal volume of 10% acetic acid solution was added to the developing solution to stop development once the microsatellite bands were visible.

Data analysis

Band patterns for each RAPD and microsatellite marker were recorded for each parent by assigning a letter to each band. Band profiles for each parent were given with 1 indicating the presence and 2 indicating the absence of a band. To avoid possible redundant information revealed by random primers, when complementary band patterns, for example, 1 1 1 2 2 1 1 1 1 2 for 1 band, 2 2 2 1 1 2 2 2 2 1 for another, were amplified by a primer, only 1 of the complementary bands was entered into the data set for genetic analysis because such a banding pattern may reflect the presence of 2 alleles at a single locus in the ten parents. Similarly, for microsatellite markers where only 2 alleles were detected among the ten parental lines, 1 of them, instead of both, was used in the genetic analysis.

Genetic distance among all 45 pairs of the ten parents were estimated from unweighted RAPD and microsatellite variant data by the Nei's distance equation (Nei 1987). Cluster analysis was based on Nei's distance and performed in the computer program NTSYS-pc using an unweighted pair group method (UPGMA).

Heterosis was evaluated with two commonly used measurements: mid-parent heterosis (calculated as a percentage of deviation from the mid-parent value) and better-parent heterosis (computed as a percentage of deviation from the better-parent value). The relationships between genetic distance and heterosis/hybrid performance were evaluated by regressing heterosis or trait values on the genetic distance in the F_1 hybrids.

Results

Polymorphisms of randomly amplified DNA markers

The number of scorable bands amplified by each primer ranged from 1 to 16. Of the 100 random 10-base primers used to amplify DNA from the ten parental lines, 74 (74%) revealed polymorphisms among the ten parents. These 74 primers generated a total of 202 non-redundant polymorphic variants (bands) with an average of 2.73 and a range of 1 to 8 bands per polymorphic primer. All parental lines had different RAPD profiles. Among the 202 RAPD variants, 21 (10.4%) were present in only one of the ten parents.

Of the 74 random primers revealing polymorphism among the ten parents, 33 generated subspecies-specific bands. Of these RAPD primers 13 gave bands unique to the six *indica* parents, 14 amplified bands specific to the four *japonica* parents and 6 yielded bands that appeared to be a codominant pattern with a set of bands specific to the *indica* parents and another set unique to the *japonica* parents. These putative subspecies-specific RAPD bands may represent alleles present at a higher allele frequency in *indica* and *japonica* subspecies, respectively.

Polymorphisms of microsatellite markers

All 23 microsatellite markers detected polymorphism among the ten parents. These primer sets revealed 90 alleles among the ten parents at the 23 loci, including 2 at 1 locus, 3 at each of 6 loci, 4 at each of 11 loci, 5 at each of 4 loci and 6 at 1 locus, giving an average of 3.91 allelic variants per locus. Among the 90 alleles, 30 (33.3%) were detected in only one of the ten parents. Unique SSR profiles could be generated for each of the ten parental lines by amplification of as few as three primer sets.

Among the 90 microsatellite alleles, 3 (3.33%) were unique to the six *indica* parents, 6 (6.67%) were specific to the four *japonica* parents. Like the RAPD markers described above, these simple sequence length polymorphisms (SSLP) may represent alleles present at a higher frequency in *indica* or *japonica* subspecies, respectively.

Genetic distances among parents

Nei's genetic distances were computed for all 45 combinations of the ten parents based on 291 non-redundant marker variants (202 RAPD variants, 89 SSR allele variants) and are presented in Table 1. The distances among the ten parents ranged from 0.0184 (XQ vs CT) to 0.1225 (NP vs IR), with an average of 0.0785 across all 45 pairs. The average genetic distance between *indica* parents and *japonica* parents was 0.1072, with a range of from 0.0975 (CE vs RG) to 0.1225 (NP vs IR). The distance with *indica* parents ranged from 0.0184 (XQ vs CT) to 0.0671 (MH vs CT), with an average of 0.0497. Within *japonica* parents, the distance ranged from 0.0192 (JL vs NP) to 0.0475 (CB vs RG), with a mean of 0.0356. These results indicate that the *indica* parents are more divergent than the *japonica* parents used in this study.

Clustering of parental lines

Cluster analysis based on the 291 marker variants (202 RAPDs and 89 SSLPs) resolved the ten parental lines into two major groups that can be considered as *indica* and *japonica*, the two major subspecies of *O. sativa* (Fig. 1). Within the *japonica* group, NP, JL and CB were grouped more closely than RG. Within the *indica* cluster, two subgroups were apparent: CT and XQ, CE, IR and R4. Line MH was most distantly related to the other *indica* lines.

Dendrograms were also constructed on the basis of either 202 RAPD or 89 SSLP variants and compared

Table 1 Genetic distances among the ten parents as calculated from the 291 marker variants

	XQ	MH	IR	CE	R4	CT	CB	JL	NP
MH	0.0597								
IR	0.0542	0.0583							
CE	0.0446	0.0641	0.0404						
R4	0.0561	0.0526	0.0414	0.0303					
CT	0.0184	0.0671	0.0564	0.0453	0.0567				
CB	0.1088	0.0985	0.1202	0.1122	0.1054	0.1083			
JL	0.1078	0.1012	0.1194	0.1057	0.1015	0.1086	0.0283		
NP	0.1145	0.1073	0.1225	0.1078	0.1037	0.1141	0.0285	0.0192	
RG	0.1011	0.1037	0.1032	0.0975	0.1001	0.0993	0.0475	0.0458	0.0442

Genetic distance is according to Nei (Nei 1987)

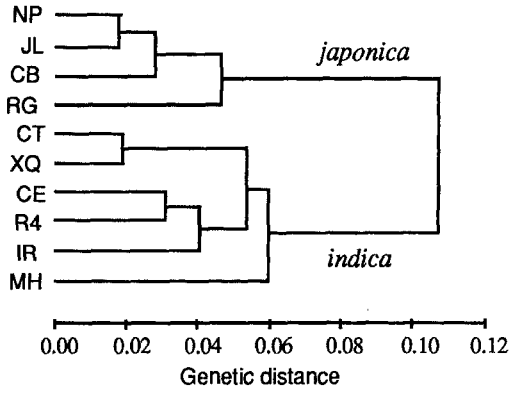

Fig. 1 A dendrogram of the ten parental lines based on 291 (202 RAPDs and 89 SSRs) marker variants. Genetic distance is according to Nei (Nei 1987)

with the dendrogram generated from the entire data set of RAPDs and SSLPs. The RAPD-based dendrogram had the same clustering structure as the dendrogram developed from the entire data set. The SSLP-based dendrogram was the same in assigning the parents into two major groups and quite similar in forming subgroups within the major groups. The only discrepancy was that while CE, IR and R4 formed one cohesive subgroup in the dendrogram based on the entire data, CE was outside the subgroup formed by R4 and IR in the dendrogram constructed with SSLP data. These results suggest that the 23 microsatellite markers used in this study provide a reliable way of assigning genotypes to different heterotic or subspecific groups.

Hybrid performance and heterosis

Two F_1 hybrids (JL × MH and NP × IR) did not head (flower) during the growing season of 1994 and therefore field data on these 2 hybrids was not available. The means and ranges of performance and heterosis of the remaining 43 F_1 hybrids are shown in Table 2. The degree of heterosis varied considerably from trait to trait. Yield potential exhibited the highest heterosis among the nine traits examined, followed by spikelets per plant, spikelets per panicle, plant height, panicle length, 1,000-grain weight. The flowering traits (days to heading and days to maturity) showed significant negative heterosis. These observations are consistent with a report by Xiao and Yuan (1988) except that significant positive heterosis was observed for flowering traits in that study. This discrepancy could be due to the use in the current study of parental lines with a consistently shorter growth duration.

The degree of heterosis for a given trait differed greatly from hybrid to hybrid. Yield potential, for example, ranged from −8.0% (XQ × CT) to 205.0% (RG × IR) for mid-parent heterosis; from −16.6% (CB × JL) to 134.2% (RG × IR) for better-parent heterosis.

Table 2 Means and ranges of hybrid performance and heterosis in the 43 F_1 hybrids (*MP* mid-parent, *BP* better-parent)

Trait	Performance		MP heterosis (%)		BP heterosis (%)	
	Mean	Range	Mean	Range	Mean	Range
Days to heading	66.4	55.0–84.0	−5.5	−20.1–47.8	−13.2	−26.5–46.6
Days to maturity	105.2	93.0–124.0	−3.3	−20.1–19.8	−9.4	−24.2–19.2
Plant height	117.7	91.9–145.1	22.2	−4.3–43.7	16.3	−9.4–40.6
Panicle length	22.8	16.7–26.3	15.2	−2.1–31.0	8.2	−7.4–28.2
Panicles per plant	7.9	4.2–12.7	6.8	−30.0–45.1	−9.8	−48.8–32.0
Spikelets per panicle	138.1	64.9–237.9	39.1	−9.6–98.5	23.4	−26.2–95.8
1,000-grain weight	26	22.6–28.6	8.6	−4.6–20.6	3.7	−11.3–20.5
Spikelets per plant	1058.5	597.4–1542.0	54.6	−7.7–173.1	33.7	−22.5–106.6
Yield potential	27.7	14.0–39.5	68.5	−8.0–205.0	42.6	−16.0–134.2

Relationship of genetic distance to hybrid performance and heterosis

The correlations of genetic distance with the performance and heterosis of the 43 hybrids were estimated by regressing heterosis or trait values on the genetic distance. As shown in Table 3, genetic distance was correlated with hybrid performance and both mid- and better-parent heterosis ($P < 0.01$) for yield potential, spikelets per plant and plant height. Genetic distance was also correlated with both performance and mid-parent heterosis for all yield potential components (panicles per plant, spikelets per panicle and 1,000-grain weight). Moreover, genetic distance was correlated with better-parent heterosis for panicles per plant and spikelets per panicle. The flowering traits (days to heading and days to maturity) showed no association with distance for performance nor with mid- or better-parent heterosis.

The usefulness of molecular markers in predicting the performance and heterosis of intra-subspecific and inter-subspecific hybrids was compared by separating the 43 hybrids into three subgroups: 22 *indica* × *japonica*, 15 *indica* × *indica*, and 6 *japonica* × *japonica* hybrids. In all three subsets, significant genotypic variation was observed for all of the traits examined within the subsets.

Regression analysis revealed significant ($P < 0.05$) correlations of genetic distance with yield potential and its heterosis within *indica* × *indica* hybrids and within *japonica* × *japonica* hybrids, and the correlations of genetic distance with yield potential and its heterosis were not significantly different from zero within *indica* × *japonica* hybrids (Table 4).

Table 3 Correlations of genetic distance with performance and heterosis in the 43 F_1 hybrid (*PF* performance, *MPH* mid-parent heterosis; *BPH* better-parent heterosis)

Trait	PF	MPH	BPH
Days of heading	0.133	0.177	0.084
Days to maturity	0.114	0.178	0.069
Plant height	0.744	0.757	0.736
Panicle length	0.368	0.590	0.721
Panicles per plant	0.430	0.691	0.505
Spikelets per panicle	0.357	0.538	0.317
1,000-grain weight	0.326	0.474	0.272
Spikelets per plant	0.785	0.724	0.724
Yield potential	0.777	0.725	0.702

$r = 0.304$ at $P = 0.05$; $r = 0.393$ at $P = 0.01$

Discussion

The results from the microsatellite analysis of ten rice inbred lines indicate that the number of alleles detectable in rice by microsatellites is very high, which is consistent with results of previous reports (Panaud et al. 1996; Wu and Tanksley 1993; Zhang et al. 1995). All of the 22 microsatellite markers, which map to 23 loci distributed in the rice genome, revealed polymorphism among the ten parents. The number of alleles revealed per microsatellite ranged from 2 to 6 among the ten parents used in this study, with an average of 3.91 per locus.

Of the 100 random 10-base primers used, 74 (74%) revealed polymorphism among the ten elite parental lines, for a total of 202 RAPD markers. The other 26 (26%) primers were monomorphic and not informative. Of the 202 bands, 21 (10.4%) occurred in only one of the ten parents. The ten parental lines could be distinguished from another using the RAPD patterns generated by as few as 2 selected random primers. These results suggest that RAPDs are potentially useful in rice variety protection and identification.

Indica and *japonica* are the two major subspecies of rice (*Oryza sativa* L.). They have different morphological characteristics and chemical and physiological features that have been widely used in classical taxonomy to assign rice accessions into appropriate subspecies. The RAPD analysis of ten parental lines in this study revealed 39 bands present exclusively in the six *indica* parents or the four *japonica* parents. Moreover, a "codominant-like" RAPD pattern with 1 band unique to the six *indica* parents and 1 band specific to the four *japonica* parents was amplified by each of six 10-base primers. Whether these "specific" bands represent alleles present either at a higher allele frequency or exclusively

Table 4 Correlations of genetic distance with performance and heterosis within subspecies and between subspecies (*PF* performance, *MPH* mid-parent heterosis, *BPH* better-parent heterosis)

Trait	Within *indica* subspecies			Within *japonica* subspecies			Between subspecies		
	PF	MPH	BPH	PF	MPH	BPH	PF	MPH	BPH
Days to heading	−0.197	−0.653**	−0.584*	0.415	−0.204	−0.388	0.281	0.179	0.004
Days to maturity	−0.157	−0.551**	−0.528*	0.168	−0.159	0.075	0.169	0.164	0.003
Plant height	0.657**	0.373	0.138	0.712	0.823*	0.802	0.065	−0.138	0.037
Panicle length	0.654**	0.627*	0.232	0.779	0.861*	0.781	−0.398	−0.331	−0.275
Panicles per plant	0.447	0.489	0.557*	−0.244	0.476	0.257	0.235	−0.231	−0.015
Spikelets per panicle	−0.044	0.248	0.044	0.809	0.869*	0.772	−0.436*	−0.522*	−0.560**
1,000-grain weight	0.534*	0.398	0.046	0.799	0.749	0.721	−0.190	0.267	0.366
Spikelets per plant	0.462	0.517*	0.457	0.901*	0.846*	0.753	−0.273	−0.416	−0.171
Yield potential	0.518*	0.541*	0.549*	0.886*	0.837*	0.761	−0.329	−0.342	−0.071

*, ** Significant at the 0.05 and 0.01 probability level, respectively

in *indica* or *japonica* subspecies needs to be confirmed with larger samples of rice germplasm.

The parents CE, R4 (IRRI relatives) and IR (IRRI variety) were tightly clustered. The parent JL was derived from NP, a *japonica* variety widely used in Japan and North China. The molecular analysis of RAPD and SSLP markers indicated that these two lines are closely related. Therefore, the grouping of the parents based on RAPD and SSR markers is consistent with classical taxonomy and pedigree information. This demonstrates the usefulness of RAPDs and SSRs in assigning rice germplasm into appropriate subspecies or variety groups.

The results from the current study demonstrates that the associations of marker-based genetic distance measures with hybrids performance and heterosis depend on the type of hybrids studied. Great differences in such association were found for (1) intra-subspecific hybrids; (2) inter-subspecific hybrids; (3) mixtures of both, as is commonly the case in diallel crosses among parental lines from *indica* and *japonica* subspecies. A similar result was observed in maize when using parental lines from flint and dent heterotic groups (Melchinger et al. 1992).

Genetic distance, measured by the 291 non-redundant pieces of information amplified by the 74 random 10-base primers and the 22 microsatellite primer sets, showed a significant ($P < 0.05$) correlation with yield potential and its heterosis for intra-subspecific hybrids, suggesting that parental genotyping based on RAPDs and SSRs could be an useful way of reducing the field work associated with making cross and hybrid field testing when attempting to identify intra-subspecific hybrids possessing high yield potential as targets for the transfer of key genes in rice. Results from a diallel set of 28 *indica* × *indica* crosses by Zhang et al. (1994, 1995) using mainly RFLPs suggest RFLP analysis as a potential means for predicting heterosis of *indica* × *indica* hybrids.

In summary, the results from the current study indicate that RAPDs and microsatellites offer a reliable and effective means of assessing genetic variation and thus provide an alternative avenue for predicting performance and heterosis of intra-subspecific hybrids.

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