

# RAPD Analysis of Genetic Diversity in Indian Land Races of Rice (*Oryza sativa* L)

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The study aims at determining genetic diversity in a set of land races in comparison to a representative sample of improved rice varieties, using random amplified polymorphic DNA (RAPD). Analysis of 36 accessions using 10 arbitrary decamer random primers, revealed 97.16% polymorphism. Similarity values among the land races ranged from 0.58 to 0.89 indicating wide diversity. The extent of diversity of the accessions was assessed using heterozygosity arithmetic mean value in land races (0.71) and cultivars (0.54). Marker Index (MI) revealed higher values in land races (120.83) than improved varieties (51.74). The land races and improved varieties formed separate clusters at 0.65 similarity suggesting that genetically distant land races could be potentially valuable sources for enlarging and enriching the gene pool of improved varieties.

**Key words :** *Oryza sativa* L, land races, varieties, RAPD, diversity.

Rice (*Oryza sativa* L) is one of the few crop plants with rich genetic diversity. The land races and wild/weedy species together constitute 80% of the rice germplasm collection. The large variability for complex quantitative traits in these accessions remains unexploited. The genetic potential of these still untapped resources could be unique and different from that of the improved varieties which have a narrow genetic base (1). There are very few reports on the assessment of genetic diversity of Indian land races using molecular markers and they deal with only scented germplasm of rice (2,3).

Genetic diversity is studied at the molecular level using various markers such as restriction fragment length polymorphism (RFLP), microsatellites, inter simple sequence repeats (ISSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) etc (4 - 7). Among these the simplest and widely used is RAPD, which allows a large number of markers to be assayed relatively inexpensively, using the polymerase chain reaction (PCR) technique and single oligonucleotide primers of arbitrary sequence (8,9). In rice, RAPD has been successfully used to assess phylogenetic relationships and to discriminate intraspecific groups of *Oryza sativa* germplasm (10 - 12). These studies indicate that *indica* is genetically more diverse than *japonica*, but both the varietal groups have a narrow genetic base(10,

13 - 15). The present study assesses the genetic diversity in land races from two major centers of genetic diversity in India, in comparison with high yielding cultivars using RAPD markers.

## Materials and Methods

A set of 22 land races from Chattisgarh (Raipur collection), five land races from north east Indian rice collection and nine high yielding semi-dwarf varieties were studied (listed in Fig. 2). DNA samples were extracted following the PVP method (16).

**RAPD analysis** — Ten decamer (OPA01 - OPA10) primers obtained from Operon Technology were used. Amplifications were performed in a reaction volume of 20  $\mu$ l containing 10 mM Tris-HCl (pH 9), 1.5 mM  $MgCl_2$ , 50 mM KCl, 0.01% gelatin, 100  $\mu$ M each of dNTPs, 10 ng of single random primer, 10 ng of genomic DNA and 1 unit of Taq polymerase (Genei, Bangalore). Amplifications were performed in a GeneAmp PCR System 9700 (PE Applied Biosystems), programmed for 5 min at 94°C, 40 cycles of 1 min at 92°C, 1 min at 35°C and 2 min at 72°C and final extension for 3 min at 72°C. The amplified products were loaded in 2% agarose gels, containing 0.5mg ml<sup>-1</sup> of ethidium bromide. The amplifications were checked for their reproducibility. The gels were documented using ImageMaster VDS Software (Pharmacia Biotech).

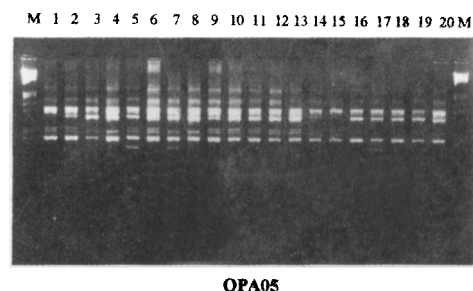
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**Statistical analysis** — Gels were scored for the presence (1) or absence (0) of bands and data analyzed using the NTSYS-pc version 2.0 (17). Indices of similarity were calculated using simple matching coefficient to estimate relationships between accessions. The average similarity was calculated for all pairwise comparisons. Cluster analyses were based on similarity matrices, using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and relationships between accessions were visualized as dendrogram. For dominant markers such as RAPD, where only two states [presence (1) or absence (0)] can be distinguished at each band position, it was assumed that each band position corresponded to a locus with two alleles, presence and absence of the band respectively. Thus each band was considered as an allele. Expected heterozygosity for land races and varieties was computed from the square sum of allele frequencies (18). The arithmetic mean heterozygosity and Marker Index were calculated according to Powell *et al* (19). The Effective Multiplex Ratio ( $EMR = n_p \beta$ ), is the number of polymorphic loci in the germplasm of interest that were analyzed in each experiment and  $\beta (n_p / n_p + n_{np})$  is the fraction of polymorphic loci. Marker Index (MI) is the product of expected heterozygosity ( $H_{av(p)}$ ) and effective multiplex ratio.  $MI = EMR H_{av(p)}$

## Results and Discussion

The extent of genetic diversity in the land races in comparison to that in the improved high yielding varieties was assessed using two methods *viz.*, (i) overall level of polymorphism among accessions as estimated from mean heterozygosity ( $H_{av}$ ) and (ii) degree of relatedness by clustering on the basis of similarity matrices.

In all 212 reproducible bands ranging from 3.9 kb to 0.25 kb were observed. The average number of bands per genotype was 6.92 in the land races and 15.66 in improved varieties. The most frequent bands in the land races were also found as the most common bands at their respective positions in the improved varieties (Fig. 1). Earlier studies on genetic variation in Asian land races using microsatellite markers indicate relatively more number of alleles in land races than in improved cultivars (20). The higher number of bands observed in the improved varieties in the present study may be due to the involvement and contribution of very large number of parental lines in the evolution of elite cultivars. For example the variety IR36 involves around 24 genotypes from six countries in its



**Fig. 1.** RAPD patterns of land races and varieties of rice. M: Molecular marker, lanes 1-10: Land races from Chattisgarh region, lanes 11-15: Land races from North East region, lanes 15-20: improved high yielding varieties.

genealogy. Being a multilocus random marker, RAPD reaction may be amplifying more number of bands in the modern varieties, with the availability of more priming sites. On the other hand, microsatellite markers use the conserved DNA sequences flanking the simple sequence repeats as primers, which are designed as specific for a locus, thus amplifying mostly a single locus.

Estimates of diversity based on allele frequency were higher in land races (average heterozygosity, 0.71), as compared to the cultivars (0.54). Similar high values of mean heterozygosity were earlier reported in the study of 42 elite rice cultivars using 8 random primers (0.80) and also in the study of 18 rice accessions, using 24 random primers (0.63) (15, 21). In another study, with 42 accessions from isozyme groups I, II and IV, an average heterozygosity value of 0.33 was obtained using four random primers (22).

Marker Index (MI), the product of expected heterozygosity and multiplex ratio was used to compare the land races and the cultivars. Distinct differences in the values were observed between land races (120.83) and improved varieties (51.74). MI is used to evaluate and compare overall utility of different markers (19). The high value of MI in land races reflects higher number of polymorphic bands detected in each band profile and higher heterozygosity in land races as compared to that in improved varieties. Heterozygosity values do not appear to be associated with number of bands as improved varieties characterized with higher number of bands show lower heterozygosity than the land races.

Of the total 212 bands, 97.16% were polymorphic with similarity values ranging from 0.58 to 0.89. The average

similarity values among the land races ranged from 0.70 to 0.82 with a mean of 0.76, compared to 0.76 to 0.83 similarity with a mean of 0.80 among the improved varieties. The average similarity between the land races and improved varieties was 0.65 (range 0.60 -0.69). Accessions from north east India shared 0.70 to 0.81 similarity with a mean of 0.71 with the land races from Chattisgarh region, and 0.60 to 0.65 similarity with a mean of 0.64 with the improved varieties. The maximum similarity between any two accessions (0.90) was between Swarna and Mahsuri varieties and the minimum similarity (0.58) was between Swarna and the land race INRC 10199.

The variability within the land races and improved cultivars was analyzed separately. Among the land races the polymorphism was as high as 96.23% (Table1), the average similarity values ranging from 0.65 to 0.78 with a mean of 0.72. The maximum similarity among land race accessions (0.86) was between INRC 9887 and INRC 9932 and minimum similarity (0.53) between INRC 9864 and INRC 9996. Among the improved varieties, polymorphism was 82.26%, the average similarity values ranged from 0.59 and 0.75 with a mean of 0.69. Among the varieties, the maximum similarity (0.84) was between Swarna and Mahsuri and minimum similarity (0.53) between Pusa 1266 and Jaya.

The UPGMA analysis of the RAPD data separated the 36 genotypes in our study into two clusters at 0.65 similarity (Fig. 2). The land races formed a major heterogeneous cluster while the improved varieties a minor loose cluster. The major cluster included all the 27 land races branching from each other between 0.65 and 0.89 similarity. Out of the five accessions from north eastern region analyzed, three clustered together. The minor cluster of the improved varieties branched between 0.76 and 0.90 similarity. The separation of the improved cultivars from the land races suggests existence of potentially novel genetic variation in the land races. Similar results were obtained in a study of 18 accessions of rice using RAPD. Two clusters were reported at 0.45 similarity, the first cluster

contained all the 16 commercial semi-dwarf accessions of Cuba, while the second cluster consisted of two tall Indian land races Buraratta and Kalaratta (21).

Though only 10 primers were used in the present study, they could together distinguish the land races from the improved varieties unambiguously at 0.65 similarity. This is in conformity with the earlier reports that at least 10 RAPD assay units are required to successfully discriminate genotypes (12). The clustering is also stable when the number of bands used in diversity analysis is above 100 (23). The results indicate that the land races have wide genetic diversity and are very distinct and distant from improved varieties. Very few such comparative studies have been made in the past. Earlier studies on genetic diversity in traditional Indian aromatic rice genotypes using RAPD showed 94.3% polymorphism and similarity in the range of 0.37 - 0.76 (2). A set of scented rice accessions belonging to Hansraj type, screened using random primers, revealed 95.1% polymorphism and similarity ranging from 0.25 to 0.77 suggesting thereby wide variability among the local accessions (3). The scented landraces too show a high polymorphism. Comparison of some of the Cuban rice varieties with a set of land races using RAPD markers revealed 0.73 genetic similarity among them (21).

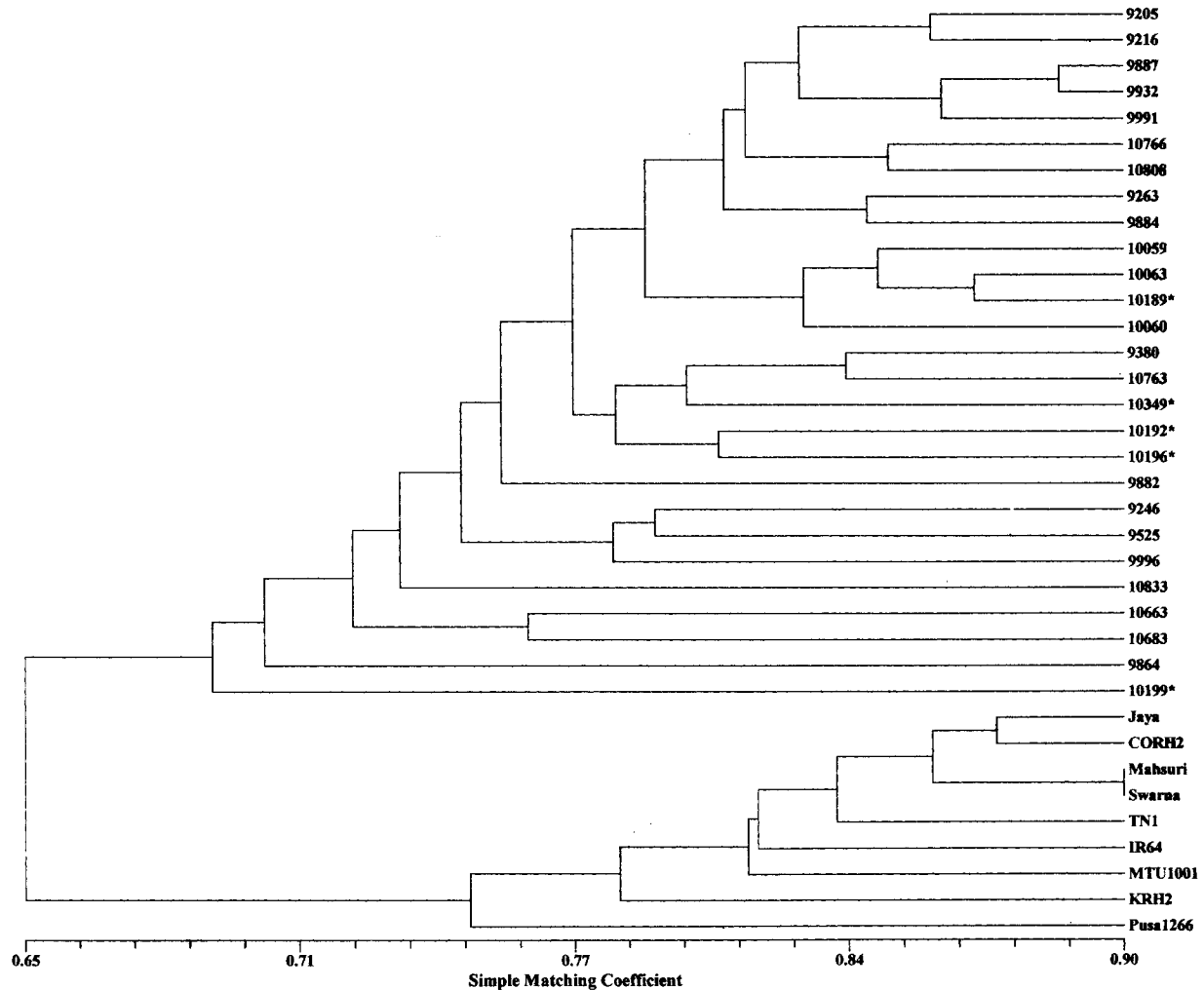
In the present study high similarity observed between improved varieties is expected as they all belong to the *indica* group, they are bred for only one ecology viz., irrigated and they share a few varieties like TN 1 or IR 8 in their parentage. Mahsuri is one of the parents of Swarna, so their high similarity is expected. Similarity values of 42 elite rice varieties which are commonly used in Indian breeding programs ranged from 0.59 to 0.95 with average similarity value of 0.83, indicating narrow genetic variability in improved varieties (15). RAPD marker-based analysis of 26 commercial rice cultivars and breeding lines showed low values of genetic distance (genetic distance = 1- genetic similarity) with the range being 0.02 to 0.66 (14). Study of

**Table 1.** Polymorphism revealed in land races and improved varieties using ten RAPD primers

	Total no. of genotypes	Total no. of bands	Average no. bands per genotype	No. of polymorphic bands	No. of monomorphic bands	Polymorphism %	( $H_{av(p)}$ ) <sup>1</sup>	MI <sup>2</sup>
Land races	27	187	6.92	178	9	96.23	0.71	120.83
Varieties	9	141	15.66	116	25	82.26	0.54	51.75

<sup>1</sup>Average expected heterozygosity for polymorphic markers ( $H_{av(p)}$ )

<sup>2</sup>Marker Index



**Fig. 2.** Dendrogram showing diversity among 36 rice accessions as derived from a UPGMA cluster analysis using simple matching coefficient based on 10 RAPD profiles. (NTSYS-PC Version 2.0). \*Accessions are from North East region.

genetic similarity in a set of 48 cultivars of *indica*, *javanica*, and Tongil (*indica/japonica*) origin using RAPD primers also ranged from 0.58 to 0.98 (12).

Rice gene pool comprising as many as 23 species including two of its progenitors is one of the richest. Its ancient origin followed by subspecific differentiation into distinct geographical groups and adaptation to diverse agro-ecological niches are, possibly, the factors that contributed

to such rich variability. Populations of wild rices as well as cultivated land races are genetically heterogeneous to varying degrees and the width of variation within a population could represent its evolutionary potential. Some of the land race populations have been reported to be more heterogeneous than annual wild populations although both are predominantly self-pollinated (24). While the north east Indian hills are on the periphery of the centre of origin of

rice, there are many secondary centers of diversity in India, which include Chattisgarh region and Jeypore tract. Among them, one unique gene centre is Chattisgarh, where the germplasm pattern changes due to extreme variation in agro-and ecoclimatic conditions. So is the case with the north east Indian hills. Despite such a rich variation existing the number of land races or progenitor species used in the improvement of rice has been low. The yield breakthrough witnessed in the mid-sixties had been through plant type based varieties tailored using the exotic dwarfing gene source DGWG. Since then breeders have been looking for new variability to further raise the ceiling to genetic yield level practically with no major breakthrough as yet, except the development of higher yielding hybrids. Knowledge of the extent of variability available within the land races and how far the variability found in them is different from that of improved varietal gene pool would be of value in parental choice in plant breeding programs. The mean heterozygosity and similarity values of the present RAPD data clearly shows that land races from centers of diversity are potential source for new allelic variation for enriching the genepool of improved varieties.

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