



Understanding allele shift using SSR markers in pedigree, modified bulk, and SSD breeding methods in rice

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Various methods of selection can be applied to advance segregating materials in autogamous crops. Under certain sets of conditions, one is preferred. But, in general, a selection system of advancing generations should be such that additive genetic variance is generated (Murty 1979). At the same time, mean performance needs to be maintained or increased in the selected progenies and the favorite genes from the donor parents should be increased. Comparative studies on different breeding methods using DNA markers are not well established.

In this study, three breeding procedures—pedigree, SSD, and modified bulk—were used to advance the materials derived from a cross between divergent rice parents Moroberekan and IR20 in an aerobic environment. The female parent of this cross is Moroberekan, an African japonica type, which was chosen because of its deep and thick roots and resistance to drought. It is relatively low yielding. The male parent is IR20, an indica type with short stature, a shallow root system, and high yield potential; however, it is susceptible to drought. The experiment was carried out at the Hebbal campus of the University of Agricultural Sciences, Bangalore, India, during the 2005 dry season. Sixty F₆ lines (20 for each breeding method) were randomly chosen from the F₅ generation and grown in the field for the different studies. Thirty-five-day-old seedlings were used for DNA extraction. DNA extraction was done following a modified C-TAB method (Cao and Oard 1997). Thirty-three SSR microsatellite markers were used to compare the three breeding methods at the DNA marker level. Based on earlier investigations, four chromosomal regions on chromosomes 1, 3, 7, and 9, which were associated with QTLs for drought resistance, grain yield, and yield component traits, were identified: RM84, RM283, RM129, RM5, RM34, RM246, RM443, RM128, RM302, RM212, and RM265 located on chromosome 1; RM231, RM545, RM546, RM7, RM218, RM251, RM563, and RM282 on chromosome 3; RM11, RM182, RM455, RM234, RM248, and RM420 on chromosome 7; and RM460, RM434, RM257, RM242, RM278, RM201, RM215, and RM205 on chromosome 9. Bands showing size similarity with Moroberekan were given a score of 1, whereas bands showing size similarity with IR20 got a score of 3; the heterozygote had a score of 2.

Analysis of molecular variance (AMOVA) was used for partitioning diversity within and among method populations using SSR marker data. This produces estimates of variance components and F statistic analogs (designated as phi-statistics, Φ_{ST}). The significance of the variance components and phi-statistics is tested using a permutation approach, eliminating the normality assumption that is inappropriate for molecular data. AMOVA was performed on Arlequin 3.0 version (Excoffier et al 1992). Genetic distance between the three breeding methods was estimated based on Nei's formula (Nei 1972). Cluster analysis to show the relatedness of the three breeding methods was done and a dendrogram was generated using the UPGMA algorithm of POPGENE version 1.31.

An AMOVA of the three breeding methods indicated that a large percentage of total genetic variation (97%) was within methods, but that a small amount (3%) was noted among methods (Table 1). The allele frequencies, by locus, in each breeding population are shown in Figure 1. There was a significant difference in allelic frequencies of the parents (Moroberekan and IR20 types) in the selection-bulk and pedigree methods, whereas the frequencies of two types of alleles were numerically different in the SSD population. This indicated a shifting of alleles in selection-bulk and pedigree populations toward the Moroberekan parent. In the SSD population, there was equal distribution of Moroberekan and IR20 allelic types. This shift in allelic frequency in the pedigree and bulk populations could be due to subsequent selection pressure across generations. Environmental changes during seasons might have contributed to the change in allele frequencies

within and between method lines; the better adapted genotypes had less competition with other lines that did not grow well under aerobic upland conditions. The literature has reported a large genetic variation within populations or species or groups and an appreciable amount among them in different plants and animals (Reeb et al 2000, Kiambi et al 2005, Vidya et al 2005).

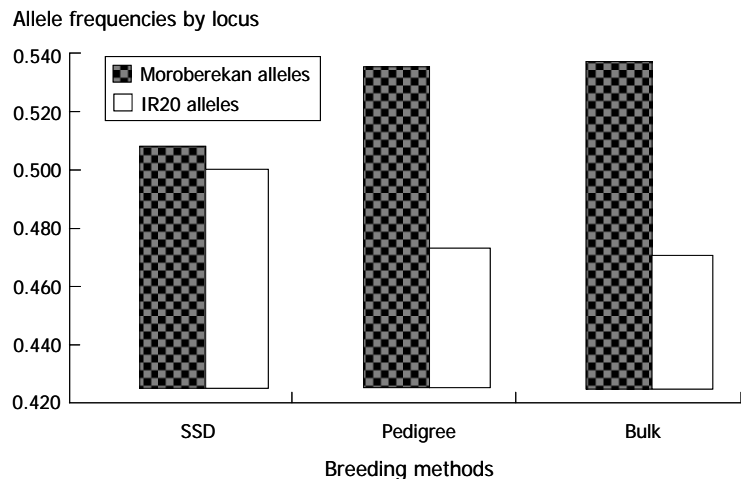


Fig. 1. Graphical comparison of allelic frequencies of SSR loci among pedigree, modified bulk, and SSD lines in the F₆ generation.

Table 1. Hierarchical AMOVA based on 33 microsatellite DNA markers, percentage of variation explained by different levels of method (subpopulation) structure, variance components, and F statistics.

SOV	DF	SS	MS	Variance components	Percentage variation	Φ -statistics	P value
Among methods	2	29.32	14.66	0.19	2.73 %	$\Phi_{ST} = 0.0273$	< 0.0099
Within methods	117	807.716	6.90	6.90	97.27 %		

Pairwise R_{ST} values differed between selection-bulk/pedigree (0.034), selection-bulk/SSD (0.016), and pedigree/SSD (0.031) (Table 2). The R_{ST} between selection-bulk/pedigree and pedigree/SSD was significant at 1% ($P = 0.010$), whereas R_{ST} between SSD and bulk was significant at 4% ($P = 0.040$). The dendrogram cluster calculated from Nei's unbiased genetic distances between method populations by the UPGMA method revealed two distinct clusters (Fig. 2). The unbiased genetic distance between the two clusters was 1.71. Cluster one comprised the bulk and SSD populations. Cluster two comprised only the pedigree population. In plant breeding, it is known that the modified bulk and SSD methods are derived from the bulk method. Thus, the chances of having undesirable alleles in the modified bulk population are greater than those in the pedigree population.

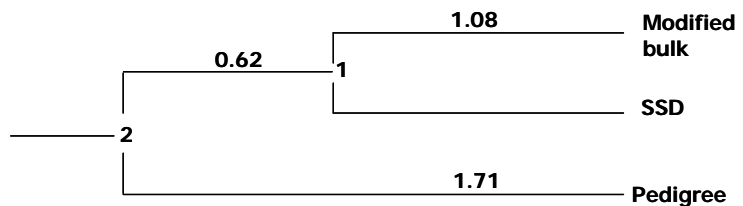


Fig. 2. Dendrogram of the relationships between three breeding method populations using the UPGMA algorithm of POPGENE.

Table 2. R_{ST} (above the diagonal line) and probability (below the diagonal line) values between method groups based on microsatellite DNA.

Method	Modified bulk	Pedigree	SSD
Modified bulk		0.034	0.016
Pedigree	0.01		0.031
SSD	0.04	0.01	

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