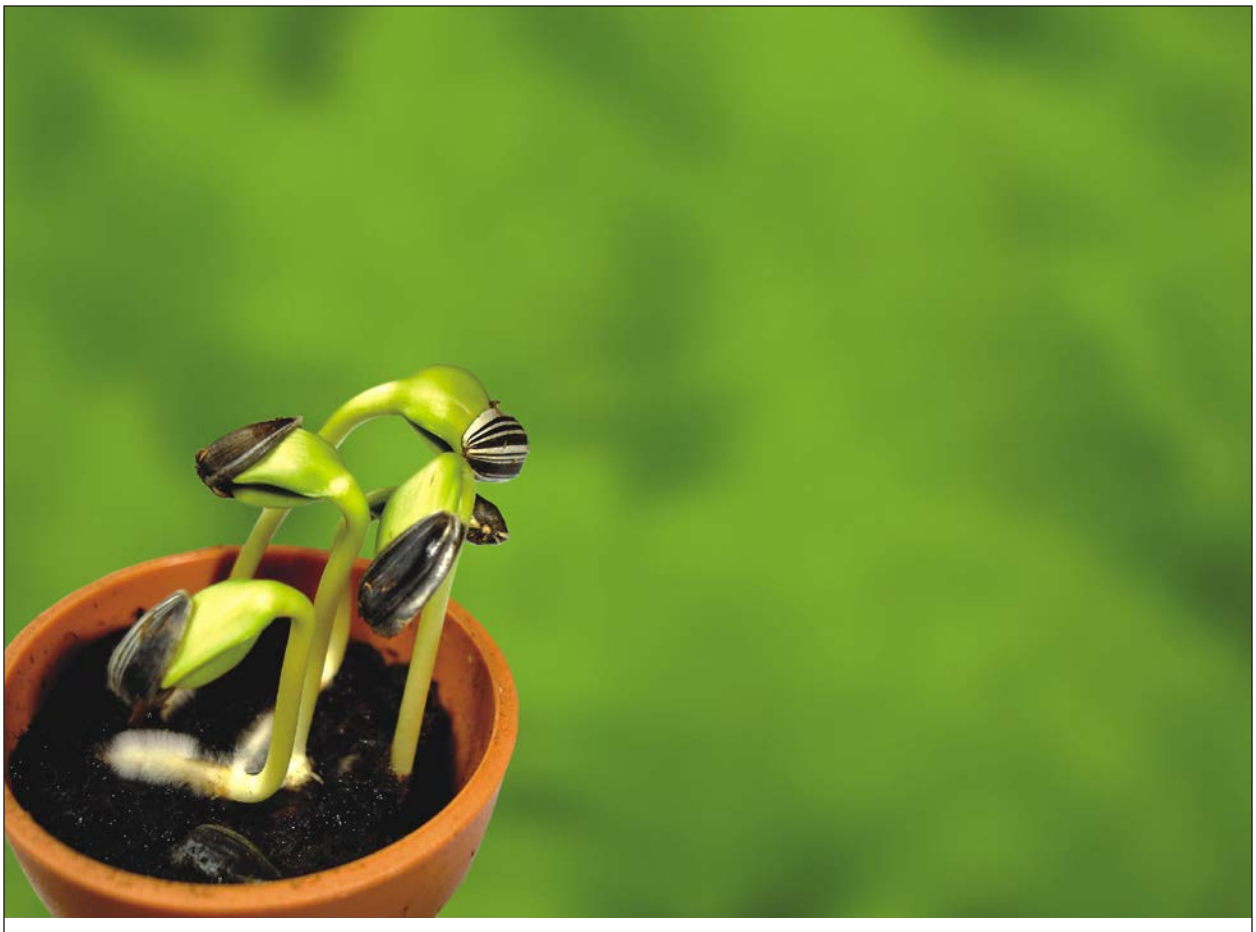

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Identification of Genotype Specific Alleles and Molecular Diversity Assessment of Popular Rice (*Oryza sativa* L.) Varieties of India

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ABSTRACT

The study was done with an aim to develop molecular tags for rice lines. A set of 29 accessions of Indian popular rice varieties was subjected to diversity study using simple sequence repeat (SSRs), a total of 87 alleles were produced that were 100% polymorphic. Twelve sets of SSR primers amplified specific alleles in 14 genotypes. The PIC value ranged from 0.57 (RM 313) to 0.98 (RM 442 and RM 163) with average of 0.78 and average genetic similarity of 0.38 was observed among the popular varieties. The maximum similarity of 0.82 was observed between Jayshree and Sarjoo52 and minimum similarity of 0.05, between Jaya and Pusa Basmati 1.Based on ecologies and duration groups showed a maximum similarity of 0.34 between IRM and RSL groups and a minimum similarity of 0.18 between IRE and RSL groups. Cluster analysis revealed PCA of rice microsatellite data from 20 primer pairs separated the four early varieties and land races from recently evolved varieties by the first and second principal component, which represent 15 and 12.2% of diversity in the sample. Out of 29 genotype, 14 genotypes produced specific alleles, which can be used as molecular tags for particular genotypes when utilized along with the non polymorphic markers in this set of genotypes it produces bar-coded molecular tags for the identification of the valuable new plant lines.

Key words: Rice, SSR marker, molecular tags, rice ecology, genetic diversity

INTRODUCTION

Rice (*O. sativa*) is the staple food of more than 60% of the world's population and about 90% of all rice grown in the world is produced and consumed in Asia. In India, rice is the most important crop, occupying about 45 million hectares of land with diverse ecological niches (Rai, 2006). Most rice varieties developed and released in India are regionally adopted as a result of development and multilocational testing of the varieties. These high yielding improved cultivars replaced the traditional cultivars in the farmer's fields. Many of these rice cultivars, however, share common ancestry and might lack genetic diversity (Shivrain *et al*., 2010). A high level of genetic diversity reduces the risk of widespread epidemics of pest and diseases (Zhu *et al*., 2000; Newton *et al*., 2009) for example of risk results from a narrow genetic base in the widespread use of resistance gene to Rice Grassy Stunt Virus (RGSV) from *O. nivara* (Cabauatan *et al*., 2009). Furthermore, narrow genetic base may be of the reasons for the lack of significant yield increase during varietal

development process. The first step towards determining the magnitude of these risks is to evaluate the genetic diversity in improved rice genotypes.

A wide range of molecular markers have been applied to genetic biodiversity studies both cultivated and wild rice such as RFLP, microsatellite, RAPD and AFLP (Singh *et al.*, 1999; Ndjiondjop *et al.*, 2006). Microsatellites are tandemly repeated nucleotide units of 1-6 bp and alleles usually differ in the no. of repeated unit. Microsatellites are generally co-dominant and highly polymorphic, even close related varieties due to variation in number of repeat units can be distinguished. Rice microsatellites are polymorphic between (Yang *et al.*, 1994; Panaud *et al.*, 1996; Akagi *et al.*, 1997) and within rice populations (Olufowote *et al.*, 1997). The data produced by Simple Sequence Repeat (SSR) analysis provide a simple fast and safe means of genome assay. The screening of microsatellite alleles in important varieties would generate a database useful for variety identification. The study was undertaken to assess the extent of genetic variation in popular rice varieties in India.

MATERIALS AND METHODS

Rice materials: The study was conducted from Jan to June 2004 at Directorate of Rice Research, Hyderabad, India. The plant materials used here in were obtained from Crop Improvement and Biotechnology Division of Directorate of Rice Research during 2004. A total of 29 popular rice cultivars comprising 25 improved and 4 land races were used in the present study. These were selected on the basis of their popularity as reported in the Production Oriented Survey (POS) (1996-2007). For precise understanding of the extent of genetic diversity among the accessions, they were initially grouped as land races and improved varieties and improved varieties were further grouped on the basis of their ecology and duration, viz., 1) Irrigated medium, 2) Irrigated early and 3) Rain fed shallow. The list of varieties taken, with their salient features are given in (Table 1).

Selection of primer and SSR assay: A total of 20 primer pairs, synthesized by M/s Research Genetics (USA) were used for PCR amplification. These primers were selected based on their uniform distribution across the 12 rice chromosomes and also on their Polymorphic Information Content (PIC) value *(as given at http://www.gramene.org)*. The sequence and details of the primers used are given in the (Table 2). The genomic DNA of different rice genotypes was isolated from rice leaves and purified following the protocol described by Dellaporta *et al.* (1983) and the quality and quantity of DNA was estimated using a UV spectrophotometer (Beckman, USA). Genomic DNA samples were diluted to 30 ng μL^{-1} and was subjected to polymerase chain. The PCR was conducted in a total reaction volume of 20 µL per sample. It contained 10X reaction buffer 2 µL (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris HCl pH 9.0), dNTPs (1.25 mM each) 2 µL, forward rice microsatellite primer (200 µM) 0.15 µL, reverse microsatellite primer (200 µM) 0.15 µL, Taq polymerase (3U μL^{-1}) 0.15 µL and DNA 50-100 µg. This reaction mixture was processed in a programmable Thermal Cycler, Gene Amp PCR System 9700 (Perkin Elmer Applied Biosystem, USA), programmed for 35 cycles for 1 min at 94°C, 1 min at 55°C, 2 min at 72°C with initial denaturation for 5 min at 94°C 4 and final extension for 7 min at 72°C. After amplification, the amplified products were mixed with 1/6th volume of the gel loading cum tracking dye (40% Sucrose: 0.25% Bromophenol) and loaded onto each well of 3% Agrose gel and run at a constant voltage of 120 volts for 3 h. The bands were visualized using Alpha Image 1220 and documented.

Table 1: Rice genotypes subjected to genetic diversity analysis and DNA fingerprinting

Varieties	Year of release	Pedigree of genotypes	Duration	Ecology	Grain type
N-22	NA	Selection From Rajbhog	90	IRE	MB
IR 36	1981	F1(IR1561/IR1737)/CR 94	84	IRE	LS
IR 64	1992	IR5657-32-2-1/IR2061-4-1-5-	90-95	IRE	LS
Rasi	1977	TN1/Co 29	90	IRE	MS
Ratna	1970	TKM 6/IR 8	84	IRE	LS
Vikas	1983/79	TKM 6/IR 8	95	IRE	MS
Tellahamsa	1971	TN1/HR-12	95-100	IRE	MS
IR 8	1966	Peta/DGWG	105	IRM	LB
TN1	NA	DGWG/TYC	100	IRM	NA
T141	1988	Mutant of Soruchinnamali	110	IRM	MS
Jaya	1968	TN1/T141	100	IRM	LB
TKM 6	NA	GEB 24/Co 18	110	IRM	NA
Basmati 370	NA	Traditional Basmati	105-110	IRM	LS
Sona	NA	GEB 24/TN1	100-105	IRM	NA
IR 20	1970	IR 262/TKM6	105	IRM	MS
Sarjoo 52	1980	TN1/Kashi	100	IRM	LB
PR 106	1978	IR8/Peta 5 /Bellapatna	100	IRM	LS
Krishnahamsa	1997	Rasi/Fine gora	90	IRME	LS
Mahsuri	1972	Taichung65/Myang Ebos 80	115-120	RSL	MS
GEB 24	-----	Land race	110-115	RSL	NA
Bahadur	1991	Pankaj/Mahsuri	125	RSL	MS
Swarna	1982	Vashishtha/ Mahsuri	125	RSL	MS
Mahalaxmi	1992	Pankaj/Mahsuri	125	RSL	MB
BPT 5204	1986	TN1/F1(Mahsuri/GEB24)	120	RSL	MS
Savithri	1983	Pankaj/Jagganath	120	RSL	SB
Ranjit	1991	Pankaj/Mahsuri	130	RSL	MS
Mandayavijaya	1986	Sona/Mahsuri	115	RSL	MS
Jayshree	1981	Jaya/Mahsuri	120	RSL	MS
PB 1	1982	Pusa150/Karnal Local	115	SCR	LS

LB = Long bold, LS = Long slender, MS = Medium slender, SB = Short bold, IRE = Irrigated Early, IRM = Irrigated Medium, SCR = Scented Rice and RSL = Rainfed Shallow

Statistical analysis: The amplified DNA fragments were scored as present (1) or absent (0) for each primer genotype combination. The data was entered into a binary matrix and subsequently analyzed using the computer package NTSYS-pc Version 2.02 (Rohlf, 1998). Dice similarity coefficients were calculated and used to ascertain the genetic interrelationship by (1) partitioning the variance of the data sets using principal component analysis (PCA); (2) Constructing phonetic tree using UPGMA (Unweighted Pair Group Method of Arithmetic mean average) cluster analysis.

RESULTS

Microsatellite polymorphism in the total sample: A total of 87 alleles were identified with these 20 primer pairs in the present analysis of 29 rice genotypes, and the banding pattern resolved by each primer pair are in accordance with single locus variation. The 20 marker loci were distributed overall 12 chromosomes except 3 and 4 of the genome. Each primer pair produced alleles ranging between 2 to 8 with an average value of 4.35. The polymorphism information content for

Table 2: List of SSR primer pairs used in the present study

Name.	Chrom.	No. of repeats	Product range	IR36 allele	PIC
RM212	1	(CT)24	112-134	136	0.719
RM424	2	(CAT)9	239-290	239	0.760
RM163	5	(GGAGA)4(GA)116(GA)20	NA	124	NA
RM508	6	(AG)17	213-235	235	0.630
RM136	6	(AGG)7	98-104	101	0.630
RM340	6	(CTT)8T3(CTT)14	119-189	163	0.670
RM405	6	(AC)14	98-110	110	0.720
RM11	7	(GA)17	123-147	140	0.828
RM404	7	(GA)33	204-240	236	0.720
RM346	7	(CTT)18	140-175	175	0.750
RM432	7	(CACT)9	167-187	187	0.690
RM475	8	(TATC)8	188-235	235	0.770
RM264	8	(GA)27	148-178	178	0.830
RM442	9	(AAG)10	257-269	252	0.690
RM442	9	(AAG)10	257-269	257	0.690
RM257	9	(CT)24	121-173	147	0.734
RM258	10	(GA)21(GGA)3	133-152	148	0.757
RM332	11	(CTT)5-12(CTT)14	162-183	183	0.650
RM101	12	(CT)37	258-334	324	0.590
RM313	12	(GT)6CA(CG)5-6-(GT)8	97-103	97	0.510
RM457	12	(TTAA)5	228-232	228	0.430

these 20 primer pairs ranged from 0.57 for RM 313 to 0.98 for RM 442 and RM 163. The results of 20 microsatellites along PIC values are given in Table 2. Out of twenty SSR markers, 12 were amplified specific alleles among 14 genotypes, six genotypes were distinguished by rest of the genotype by two markers, while rest of the 8 has amplified specific alleles using single marker only.

Genetic similarity analysis between genotype: Genetic similarity was calculated for SSR data. Among the 29 genotypes the mean genetic similarity was 0.38. The maximum amount of similarity (0.82) was observed between Jayshree and Sarjoo52 and the minimum similarity (0.05) between Jaya and PB1. Among the improved varieties, when they are grouped according to their ecologies and duration, maximum similarity (0.34) was observed between IRM and RSL groups and a minimum 0.18 was observed between IRE and RSL groups (Table 3).

Identification of genotype specific allele: Out of 29 genotype, using 20 polymorphic SSR marker, 14 genotypes produced specific alleles. The specific alleles were amplified using 12 SSR markers (Table 4). The varieties are IR8, IR36, IR64, IR20 genotypes from IRRI, highly demanding genotypes as per the breeder seed indent, Jaya and BPT5204, Genotype for Eastern UP, Sarjoo 52, highly drought tolecrance genotype N22, one traditional basmati genotype Basmati 370 and TKM6, Sona, Bahadur, Ranjit and Khamsa.

Principal component analysis and dendrogram analysis: Principal component analysis of the rice microsatellite data from 20 primer pairs separated the 4 early-evolved varieties and land

Table 3: Average genetic similarity calculated as Dice coefficients for different groups of rice genotypes based on SSR markers (Standard deviation)

	Genetic similarity between the groups			Within the group		
Groups	LR	IRM	RSL	IRE	Mean±SEM	SD(n)
LR		0.140(32)[a]	0.122(36)	0.091(32)	0.62±0.160	0.325
IRM	0.363±0.040		0.119(72)	0.115(64)	0.53±0.097	0.277
RSL	0.315±0.033	0.345±0.028		0.109(72)	0.51±0.087	0.262
IRE	0.238±0.026	0.320±0.029	0.178±0.026		0.51±0.091	0.258

[a]Figures in parenthesis are the numbers of pair wise comparisons

Table 4: Allelic distribution and informatics alleles amplified by SSR markers among 29 rice genotypes

	Total	No. of genotypes for the alleles							
SSR	No. of								
marker	Allele	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8
RM 11	4	130 (3)	135 (3)	140 (8)	145 (13)				
RM 258	4	140 (8)	145 (IR-20)	150 (4)	155 (7)				
RM 101	5	140 (10)	145 (3)	150 (13)	155 (Mahsuri)	180 (Sona)			
RM 212	4	120 (IR-64)	135 (17)	140 (5)	150 (7)				
RM 313	3	111 (11)	112 (8)	115 (9)					
RM 508	3	220 (23)	235 (IR-36)	250 (5)					
RM 475	3	235 (10)	240 (9)	245 (5)					
RM 136	4	80 (Sona)	90 (N-22)	100 (19)	110 (Basmati 370)				
RM 163	8	140 (2)	145 (Sarjoo 52)	150 (8)	155 (5)	160 (Jaya)	170 (5)	175 (3)	180 (4)
RM 340	4	100 (5)	160 (21)	170 (Jaya)	180 (2)				
RM 264	8	160 (BPT-5204)	180 (6)	190 (7)	195 (Bahadur)	200 (4)	210 (PR-106)		
RM 346	5	160 (4)	175 (7)	180 (10)	185 (9)	350 (Ranjit)			
RM 432	5	190 (8)	195 (14)	200 (BPT-5204)	210 (5)	300 (Ranjit)			
RM 424	3	220 (6)	230 (11)	240 (10)					
RM 457	2	230 (13)	235 (15)						
RM 442	5	250 (6)	255 (3)	260 (6)	265 (3)	270 (4)			
RM 405	5	110 (14)	115 (IR-64)	120 (IR-20)	125 (6)	130 (IR-8)			
RM 404	2	235 (10)	240 (16)						
RM 257	4	145 (7)	150 (8)	155 (TKM-6)	160 (6)				
RM 332	3	160 (8)	170 (14)	180 (7)					

races from the recently evolved varieties by the first and second principal component (Fig. 1). Figure 2 which represented 15 and 12.2% of the diversity in the sample. Four clusters were revealed by the first two principal components. Cluster one included three evolved varieties in addition to four land races. Cluster two included seven genotypes belonging to RSL ecology. Cluster three comprised five genotypes from IRM and IRE groups. And the fourth cluster includes four genotypes, in which 3 genotypes are of IRE type and remaining genotypes is of IRM type. Remaining six genotypes were placed in between the cluster. A dendrogram derived from UPGMA cluster analysis based on the on the dice similarity coefficient matrix for 29 accessions was constructed. The genetic similarity coefficient for all accessions ranged from 0.05-0.82 and averaged 0.38. Cluster analysis separated the genotypes into two groups at 0.28 similarity (Fig. 2). The first

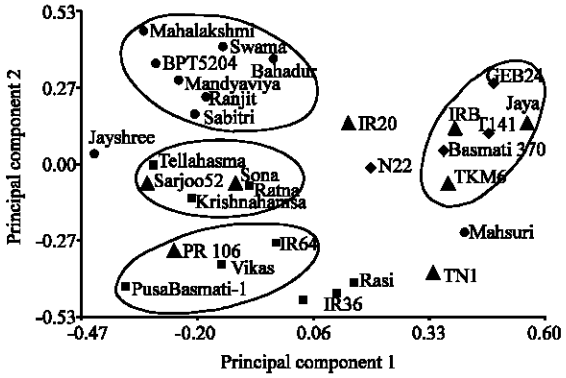

Fig. 1: Two dimensional plot of genetic diversity among the popular varieties as revealed by PCA based on microsatellite markers

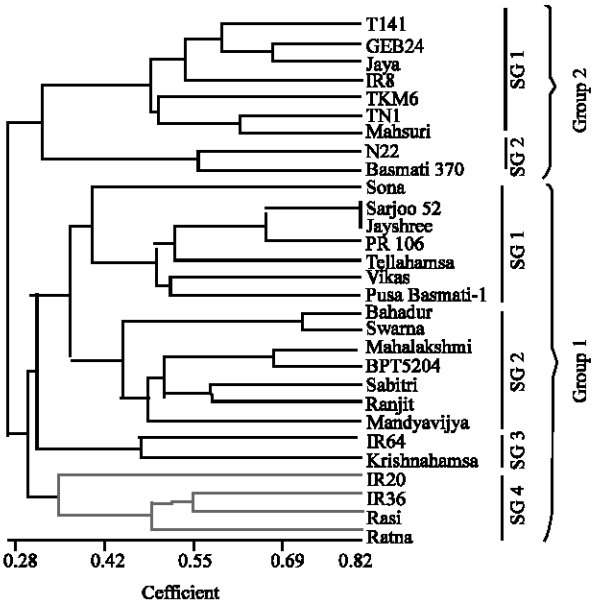

Fig. 2: Dendrogram of 29 genotypes based on 20 microsatellite markers constructed based on Dice's similarity coefficient

group comprised 9 genotypes while 7 genotypes formed sub group at 0.34 similarity, two genotypes (N22 and Basmati 370) were separated as a subgroup 2. The 2nd major group consisted of 20 genotypes. At 0.31 similarity four genotypes (IR 20, IR 36, Rasi and Ratna) formed one sub group. From the major group, another group of two genotypes (IR 64 and Krishnahamsa separated at 0.33 similarity. Remaining 14 genotypes formed two subgroups. Each with 7 genotypes, separated at 0.39 similarity.

DISCUSSION

Information and characterization of genetic diversity in the popular rice varieties would be useful in determining present trends in rice breeding and in assessing alternatives for improving current cultivars. The present study used microsatellite markers to characterize popularly grown

varieties from irrigated ecology with early and medium duration (IRE and IRM) and rainfed shallow land ecology to provide information for the maintenance or expansion of diversity in future breeding programs.

Rice microsatellite polymorphism: While allelic divergence at microsatellite loci in closely related rice germplasm usually involves changes due to expansion / contraction in the repeat length, insertion/deletion were also observed among and within species. Microsatellite survey of 29 genotypes comprising 25 improved and 4 traditional varieties with 20 primer pairs indicated a high level of genetic diversity (0.78) and the average number of alleles per primer was 4.35. As pointed out by many recent studies, microsatellite possesses hypervariability and high resolving power among various genetic markers (Powell *et al*., 1996; Davierwala *et al*., 2000). In their studies on 283 accessions of land races and cultivars of rice, Yang *et al*. (1994) detected 93 alleles with 10 SSR loci that were much larger than those detected using other types of primer pairs. Panaud *et al*. (1996) found 2-9 alleles for microsatellite primer pairs in 22 japonica and indica cultivars. Akagi *et al*. (1997) found 5-10 alleles among 59 closely related japonica cultivars. The higher level of polymorphism associated with microsatellite is to be expected partly because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Powell *et al*., 1996). These all studies support the findings of this study. The compound nature of the markers included in the present study might have also contributed to potential diversity, as the separate repeats involved are susceptible to DNA slippage and size mutations. These results also indicate that microsatellite markers are sufficiently informative and powerful to assess the genetic variability.

Genetic variability: Although, Mackill (1995) has reported in his paper that due to intensive breeding efforts, genetic diversity of modern cultivars has been reduced, in present study it has been found that there is fairly good diversity among the popular genotypes of rice. The genetic variability of the popular varieties was reflected in the polymorphism content 0.78. The popular varieties appear to possess adequate genetic diversity in the present study. Since SSR markers are known to be hypervariable the estimate is probably higher than other loci in the genome. RM 442 and RM 163 for GA and AG repeat have shown maximum PIC values of 0.98 which can be contributed to the polymorphic nature of GA and AG base repeats.

In the 25 improved varieties from the irrigated and rainfed shallow land ecologies, PIC value of 0.84 was observed. The high values of polymorphism obtained in the present study were corroborated by the observation of Sebastian *et al*. (1998) and Davierwala *et al*. (2000) in their analyses of popular rice varieties with microsatellite markers. The values of polymorphism were always higher than 70% in the studies involving improved varieties analyzed by microsatellite. Analysis of pedigree information of the improved varieties in the present study suggested that IR8, TN1, TKM6 and Mahsuri have been the most frequently used in crossing programs. The improved varieties are mostly tailored using the exotic dwarfing gene DGWG from TN1 and IR8. The improved varieties therefore represent a narrow genetic base. However, they till exhibit extensive genetic diversity for various morphological, biological and physical characters as well as at the molecular level. Among the 29 genotypes, the mean genetic similarity obtained was 0.38 indicating adequate genetic diversity in the present sample. Davierwala *et al*. (2000) also demonstrated the mean genetic similarity of 0.37 among 42 elite rice varieties with the microsatellite marker and support our findings. Among the improved varieties alone the mean genetic similarity was 0.29.

Among the four land races the mean genetic similarity was 0.42. Approximately 60% of the rice samples were clustered with less than 45% similarity. The hypervariabilty of microsatellite markers would have contributed to the high polymorphism values and it was supported by the study of Garland *et al.* (1999). They showed that the closely related Australian and US medium grain cultivars clustered to 0.49 and the closely related Australian long grain cultivars clustered with similarity values ranging from 0.60 to 0.49. Between the two ecologies with different duration also a 30% genetic similarity was observed.

Unweighted pair group method with arithmetic mean clustering: The unweighted pair group method with arithmetic mean (UPGMA) cluster analysis provided a better resolution of the relationship among the rice genotypes, which were broadly clustered into two major groups at 0.28 similarity. The first major group comprised nine genotypes while seven genotypes formed one subgroup at 0.34 similarity, two genotypes (N22 and Basmati 370) separated as group72. Though N22 and Basmati 370 belong to altogether different grops, their clustering together in the present study may be explained by the fact that their basic difference from the improved variety might have forced both of them to group together. The second major group consisted of 20 genotypes. At 0.31 similarity, four genotypes (IR20, IR30, Rasi and Ratna) formed one subgroup. From the major group two genotypes (IR64 and Krishnahamsa) separated at 0.33 similarity. Remaining 14 genotypes formed two groups, first with Mandyavijaya, Ranjit, Sabitri, BPT 5204, Mahalakshmi, Swarna and Bahadur and second subgroup with Pusa Basmati-1, Vikas, Tellahamsa, PR106, Jayshree and Sarjoo52. The grouping of the genotypes by UPGMA analysis clearly distinguished the traditional like IR8, TN1, Mahsuri from recent improved varieties which involve complex pedigree in comparison to early developed varieties. N22, a drought tolerant varieties genotype from isozyme group II grouped with Basmati 370, an aromatic genotype of Isozyme group V. The present study showed that physiological traits too play a role in grouping. As seven genotypes from rainfed shallow land ecology clustered together, though Mahsuri, a donor for rainfed shallow land ecology was not grouped along with them. It is unlikely that the samples analyzed had exactly same patterns as the individual plants involved in selection and hybridization leading the development of improved cultivars. Despite being sharing same parents Ratna and Vikas were grouping apart, which might be due to differential selection pressure and selection criteria applied for specific trait(s) Singh *et al.* (1999). The results were supported by the reports on IR varieties (IR 20, IR 36 and IR 64), which were grouped independently due to their complex parentage and several recombination events. Though Pusa Basmati 1 and Basmati 370 are aromatic rice varieties, they were not grouped together which shows that the markers used in the present study appear to be not associated with the aromatic traits.

The use of these markers in the genetic diversity studies helped in grouping the genotypes according to their genetic relatedness. The SSR markers gave more groups with fewer genotypes in each cluster. When more clusters are obtained with fewer genotypes in each cluster are obtained with fewer genotypes in each cluster, the significance in clustering is higher because of smaller genetic differences between the genotypes in a cluster. As microsatellite markers are more polymorphic and specific, the polyallelic nature of SSR markers has the advantage of discriminating the individual genotype more precisely. For example RM 163 amplified 8 alleles across 29 genotypes studied in to 8 different allelic groups. Also in the study some unique alleles were generated for same genotypes, since SSR markers detect finer level of variation among closely related lines.

Principal component analysis: The Principal Component Analysis (PCA) is one of the multivariate approaches of grouping based on the similarity coefficient (or) variance-covariance values of the component traits of the entities. It is expected to be more informative about differentiation among major groups while the cluster analysis provides higher resolution among closely related population. Overall, four distinct clusters were revealed by the first two principal components. Cluster one included six genotypes comprising three traditional varieties and three early-improved varieties that were derivatives of traditional varieties of exotic or native origin. All of the six genotypes were either simple crosses between two parents or selections without involvement of complex crosses. Cluster two included seven genotypes from rainfed shallow land ecology suggesting the grouping based on physiological traits. Cluster three and cluster four included improved varieties from irrigated ecology. The diversity among these nine genotypes appears narrow. The varieties released from IRRI viz., IR20 and IR36 were placed in between the clusters not grouping specifically which may be due to their complex parentage from diverse origins. But the exception is TN1, which has not grouped with early-developed varieties though it shares DGWG as a parent with IR8. Mahsuri also maintained its identity, as its pedigree was entirely different from that of other varieties, but the notable point here is its non inclusion in the rainfed shallow land ecology group though it was the donor of the trait.

An expectation of using one primer pair to distinguish all existing rice cultivars would be too high to be realistic. In most of the diversity studies, one most important point of consideration is the number of markers for analysis to rightly mirror the variations representative of the whole genome in order to derive reliable estimates of biodiversity. Beckmann and Soller (1986a) have estimated that 20-30 polymorphic markers would be needed to differentiate the existing inbred strains of maize. From the present study it is clear that unique SSR profiles could be generated for most of the genotypes by using a few primers sets per chromosome, since SSR markers detect finer levels of variation among closely related lines and varietal profiling based on SSR markers is more reliable. For 40 cultivars with 38 microsatellite primers, unique SSR profile was generated for each of the genotype by using as few as three primers per chromosome (Ravi *et al.*, 2003). In the present study, unique alleles were observed for 14 varieties, so further analysis with more primer pairs would generate a reliable profile for the popular varieties. Precise identification of crop cultivars is required for varietal registration, preventing misappropriation and for protection of plant breeders as well as farmers rights. While the debate is going on with regard to the suitability of DNA data for defining distinctiveness, uniformity and stability (DUS) of crop varieties and for deciding the minimum distance for declaring two varieties as different, creation of a data base on the morphological characteristics and DNA fragment patterns is widely accepted by all concerned. Neeraja *et al.* (2005) characterised a set of tall rice land races using 17 gene-derived simple sequence repeats (SSRs) and eight other genomic SSRs. Gene-derived SSRs (0.68) had lower polymorphism information content than the other SSRs (0.87). UPGMA analysis of gene-derived SSRs data separated tall landraces and high-yielding varieties, which were nitrogen responsive into a major cluster and the other three landraces, which were non9 responsive to nitrogen into a minor cluster at 0.37 similarities. The use of gene-derived markers that target functional loci appears to be an effective strategy in characterizing landraces for optimizing choice of parents for hybridization programmes.

Herrera *et al.* (2008) assessed genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers to broaden the genetic bases of rice germplasm in the country. Although the genetic diversity was low, SSRs proved to be an efficient tool in assessing the genetic diversity

of rice genotypes and presented an example in support of this study. Firstly, SSR markers are efficient and suitable for genetic relatedness studies. Secondly, SSR markers provide a more reliable and reproducible approach for genotype specific fingerprinting for cultivar identification. Thirdly, SSRs can be used to generate locus specific allelic information for use as molecular IDs for cultivars and such information can be used in assessment of seed purity using these markers. It is recommended that the database of markers derived from microsatellites should be used as a source of information on the genetic relationships of the various potential parents, so that the genetic diversity of future cultivars can be maintained at the highest practicable level. Regarding the genetic diversity of the popular varieties, though it appears adequate diversity in the studied material, further characterization with more microsatellite primers is warranted so as to attribute the observed diversity to hypervariability of the microsatellites or to the inherent diversity in the improved varieties.

CONCLUSION

This study concludes that the simple sequence markers are a sensitive and unique tool to identify/develop a molecular tag for closely related or distinct rice varieties and also useful for the registration of the new varieties. The data generated particularity for 14 popular rice varieties will be useful for true identification of varieties, in contrast morphological identification of varieties, is stage and environment specific and will take more time in comparison to molecular analysis.

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