

MOLECULAR CHARACTERIZATION OF RICE (*ORYZA SATIVA* L.) GENOTYPES RESISTANT TO MOISTURE STRESS

DIPALI A. PATEL, D. A. PATEL, SUDESHNA CHAKRABORTY*, DHARNA J. PARMAR AND N. J. JADAV
Department of Genetics and Plant Breeding, Anand Agricultural University, Anand -388 110, Gujarat, INDIA
e-mail: schakraborty.bio@gmail.com

KEYWORDS

Rice
Moisture stress
RAPD
SSR
Genetic diversity

Received on :
10.04.2014

Accepted on :
24.05.2014

*Corresponding
author

ABSTRACT

RAPD and SSR analysis of 26 genotypes of rice were performed; where RAPD and SSR with 15 and 10 primers amplified a total of 268 and 99 fragments respectively. The dendrogram generated through the study revealed that upland varieties clustered together in cluster A whereas transplanted in cluster B. The varieties with maximum similarity with other genotypes could be used as parents in hybridization programme, to generate maximum variability in segregating generation and increase scope of isolating desirable recombinants.

INTRODUCTION

Rice (*Oryza sativa* L.) is the world's most important food crop and a primary source of food for more than half of the world's population (Davla *et al.*, 2013; Reddy *et al.*, 2013). It has also become a model organism for genome analysis, having a diploid chromosome number of 24 and the smallest genome size of all major crop plants of 430 Mb. Although, rice is cultivating in large area but the final yield gain per unit area is very less due to biotic and abiotic stresses (Bala Krishna and Satyanarayana, 2013). Rice, generally grown under flooded conditions, is susceptible to drought stress owing to its shallow root distribution and limited capacity to extract water from deep soil layers (David, 1994). Yield of rainfed lowland rice, which occupies about 25% of the world's rice areas, are drastically reduced by drought due to unpredictable, insufficient and uneven rainfall during the growing period. To reduce yield losses of rice crops in rain fed lowland areas and to increase overall rice production, new rice varieties with greater adaptation to drought are essential. Hence, the development of drought resistant cultivars with a higher yield potential is one of the main objectives of rain fed lowland rice breeding programme. A critical analysis of the genetic variability is a prerequisite for initiating any crop improvement programme and for adopting of appropriate selection techniques (Dhanwani *et al.*, 2013). However, this selection process is labour intensive and slow as it requires cultivation of breeding populations under drought conditions. Marker-assisted selection (MAS) is cheaper and more convenient than phenotype-based selection and it presently is the only option to combine traits by gene pyramiding. DNA based markers

can be derived from quantitative trait loci (QTL) and allow selection already in the seedling stage (Degenkolbe *et al.*, 2013). Moreover, they allow the proper grouping of different populations and varietal groups and resolve doubts about the accession classification. This is of great advantage, because the high intra-specific variability and the environmental effects can hinder the differentiation of populations or varietal groups based only on the phenotypic evaluation (Souza *et al.*, 2013).

In this context, an attempt has been made to study the molecular characterization of rice genotypes resistant to moisture stress. Also, genotyping can be extremely useful to breeders in their efforts to manage genetic resources by helping them to identify unique materials that need to be conserved. The purpose of this study was to identify the specific primers, which are likely to be efficient in revealing the diversity among the genotypes.

MATERIALS AND METHODS

The experimental material consisted of 26 rice genotypes comprising majority of upland and some low land varieties obtained from Main Rice Research Station, Anand Agricultural University, Nawagam (Table 1). Seedlings of all the genotypes were raised in pots. Fresh leaves were collected and further utilized for isolating genomic DNA to study genetic diversity and polymorphism by RAPD and SSR analysis. The DNA was extracted from the fresh leaves of four weeks old seedling by CTAB method as described by Ahmadikhah *et al.* (2007) with some modifications. To estimate quantity and quality (in terms of protein and RNA contamination) of isolated genomic DNA, spectrophotometry was performed and data were analyzed

using Nanodrop N.D.1000 software (ver.3.3.0). The RAPD analysis was performed according to Williams *et al.*, (1990) with minor modifications. The PCR reaction steps for RAPD are 94°C for 5 minutes (Initial denaturation), 45 cycles each of 94° for 1 minute (Denaturation), 38°C or 40°C for 1 minute (Primer annealing), 72°C for 2 minutes (Extension of annealed primer) and 72°C for 10 minutes (Final Extension). For SSR analysis the PCR condition consisted of Initial denaturation at 94°C for 7 minute, with 45 cycles of final denaturation at 94°C for 45 second, annealing at 55°C for 1 minute and extension at 7 min, followed by final extension at 72°C for 7 min. The amplified products for RAPD and SSR were analyzed electrophoretically using 1.8 % and 2.5% agarose gel, respectively. The separated bands were visualized under UV transilluminator and photographed using syngene gene snap-G-box (Alpha Ease FC4.0.0 gel Documentation system). Each amplified product was scored across all the genotypes for its presence or absence. The scores 1 and 0 indicate the presence or absence of bands, respectively. The data were entered into binary matrix and subsequently analyzed using NTSYSpc version 2.02. Coefficients of similarity were calculated as Jaccard's similarity coefficient by SIMQUAL subroutine in SIMILARITY routine. The matrix of similarity was clustered using UPGMA algorithm under Sequential Agglomerative Hierarchical Nesting (SHAN) module of the NTSYS pc. Relationships among rice cultivars were graphically represented in the form of dendrograms. The cophenetic correlation analysis was carried out using COPH function of NTSYS pc. and dendrogram constructed based on the similarity coefficients. The PIC value for each locus was calculated on the basis of allele frequency (Anderson *et al.*, 1993).

RESULTS AND DISCUSSION

The present investigation was carried out in the Biotechnology Laboratory of Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, to analyze the genetic diversity among 26 rice genotypes using RAPD and SSR markers.

In all, 60 different RAPD primers were used in the present study, out of which 15 produced polymorphic results (Table 2). The results obtained revealed different degree of polymorphism by different primers. Amplification of total

genomic DNA from different varieties produced a total of 268 fragments, of which 267 (99.62%) were polymorphic in nature. The percentage of polymorphic bands shown by different primers ranged from 94.44 (OPC-8) to 100 (all except OPC-8). One of the reasons for this high level of polymorphism could be due to extensive intra-specific variation in rice. Although the majority of primers produced polymorphic bands, no single primer could clearly distinguish all the genotypes. RAPD marker OPC-15 produced maximum numbers of 247 bands, while OPK-18 amplified minimum numbers of 115 bands. The PIC values ranged from 0.875 (OPK-18) to 0.938 (OPK-20) with an average of 0.918 (Table 3). The similarity coefficient values ranged from 0.16 to 0.61 (Table 4). This indicated a fair range of variability in the similarity coefficient values, suggesting a fairly wide genetic base of 26 rice genotypes used in the experiment. The highest value of similarity coefficient (0.61) was found between the varieties GR12 and IR-64 (0.61) and between the varieties Lalat and Tapaswini (0.61). However, the lowest value of similarity coefficient (0.16) was observed between the varieties GR-5 and Tapaswini. In order to analyze the relatedness among the genotypes studied, the UPGMA-based dendrogram was constructed using paired matrix values for pooled RAPD data. Dendrogram generated for 15 RAPD primers against 26 rice genotypes formed two major clusters, 'A' and 'B' (Fig 1). Major cluster 'A' was further divided into two sub-clusters, 'A1' and 'A2'. Sub-cluster 'A1' consisted of three varieties *viz.*, Sathi 34-36, GR-5 and GR-9 (all upland cultivars), while sub-cluster 'A2' comprised of ten varieties *viz.*, SK-20, Ashoka -200F, GR-8, AAUDR-1, DDR-22, Kalinga-III, Vandana, Annanda, GR-3 and GR-4. Cluster "B" was also divided into three sub-clusters, 'B1', 'B2' and 'B3'. Sub-cluster "B1" comprised of six genotypes *viz.*, GR-6, GR-11, GR-7, GR-12, IR-64 and Jaya. The subcluster 'B2' comprised of other six genotypes *i.e.* Swarna, TN-1, Lalat, Tapaswini, Pusa Basmati-1 and IET-18990, whereas sub-cluster 'B3' consisted of only one variety *i.e.* Samba Masuri. Most of cultivars included in cluster 'A' were upland varieties, while cluster 'B' comprised mostly transplanted varieties. The results revealed a moderate level of genetic variation among rice genotypes and led to the establishment of genetic relationships between them. The similarity matrix and dendrogram revealed that the upland paddy varieties *viz.*, Sathi 34-36, GR-5, GR-9, SK-20, Ashoka-200F, GR-8 and AAUDR-1 are grouped into major cluster A, indicating that these genotypes may have similar genetic constitution and expression profiles for moisture stress. The varieties, GR-12 and IR-64 showed highest similarity in cluster B because of IR-64 is one of the two parents of GR-12. Tapaswini and Lalat showed minimum similarity with all the other genotypes, which can be used as parents in hybridization programme so as to generate maximum variability in segregating generations and

Table 1: List of rice genotypes taken in the study

Sr. No.	Genotypes	Sr. No.	Genotypes
1.	Sathi 34-36	14.	GR-6
2.	SK-20	15.	GR-7
3.	GR-5	16.	GR-11
4.	GR-8	17.	GR-12
5.	GR-9	18.	Jaya
6.	Ashoka-200F	19.	Samba Masuri
7.	AAUDR-1	20.	IR-64
8.	DDR-22	21.	Swarna
9.	Kalinga-III	22.	Lalat
10.	Vandana	23.	TN-1
11.	Annanda	24.	Tapaswini
12.	GR-3	25.	Pusa Basmati-1
13.	GR-4	26.	IET-18990

Table 2: List of RAPD primers showed polymorphic results

Sr. No.	Name of Primer	Sr. No.	Name of Primer	Sr. No.	Name of Primer
1.	OPA-2	6.	OPC-6	11.	OPK-8
2.	OPA-7	7.	OPC-8	12.	OPK-16
3.	OPA-8	8.	OPC-8	13.	OPK-17
4.	OPA-10	9.	OPC-11	14.	OPK-18
5.	OPC-2	10.	OPC-15	15.	OPK-20

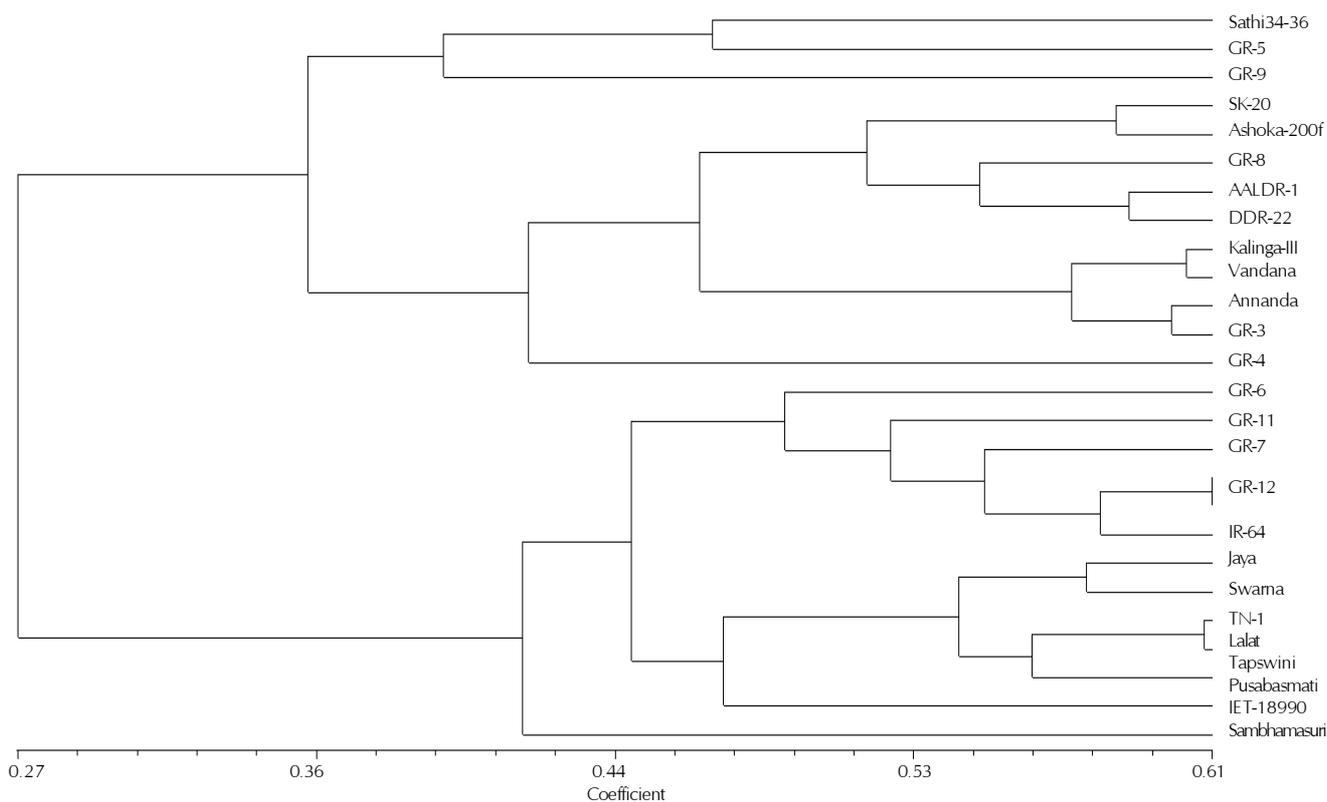


Figure 1: Dendrogram of genetic relationship among rice genotypes based on RAPD markers.

Table 3: Analysis of RAPD patterns generated using 15 arbitrary primers for rice genotypes

Sr. No.	Name of Primer	Maximum scorable bands	Polymorphic loci (P)	Total Loci (T)	Percentage polymorphism	PIC value
1.	OPA-2	222	21	21	100	0.927
2.	OPA-7	135	19	19	100	0.928
3.	OPA-8	190	18	18	100	0.920
4.	OPA-10	178	22	22	100	0.933
5.	OPC-2	166	18	18	100	0.929
6.	OPC-6	162	19	19	100	0.930
7.	OPC-7	132	15	15	100	0.909
8.	OPC-8	190	17	17	94.44	0.914
9.	OPC-11	152	19	19	100	0.927
10.	OPC-15	247	20	20	100	0.934
11.	OPK-7	200	16	16	100	0.920
12.	OPK-16	164	15	15	100	0.909
13.	OPK-17	142	13	13	100	0.882
14.	OPK-18	115	13	13	100	0.875
15.	OPK-20	238	22	22	100	0.938
	Range	115-247	13-22	13-22	94.44-100	0.875-0.933
	Average	175.53	17.8	17.8	99.62	0.918
Pooled		2634	267	267	-	-

increase scope of isolating desirable recombinants. On the whole, RAPD analysis revealed very useful information about genetic variability among cultivars, which can be used by plant breeders in their breeding programme. The present result is in accordance with the results of Islam *et al.* (2013) where genetic diversity of drought tolerant rice were carried out through RAPD analysis. Also, Ogunbayo *et al.* (2005) reported differentiation among rice genotypes was higher for RAPD

markers than for morphological classification.

All the 26 rice genotypes were further analyzed by using 10 trait-specific SSR markers for genetic diversity analysis. Total 99 amplified products were obtained. The allele length for these 10 SSR markers varied from 80 to 238 bp. The highest allele length was recorded for RM 242 (238bp) for varieties Vandana and GR-3. The allele frequency produced by different markers was observed in the range of 0.038 to 0.500. The

Table 4: Similarity matrix of 26 rice genotypes based on RAPD markers

	Sathi 34-36	sk-20	GR-5	GR-8	GR-9	Ashoka-200F	AAUDR-1	DDR-22	Kalinga-III	Vandana	Annanda	GR-3	GR-4	GR-6	GR-7	GR-11	GR-12	Jaya	Samba masuri	IR-64	Swama Lalat	Tapaswini	Pusa basmati-1	IET-18990		
Sathi34-36	1																									
sk-20	0.47	1																								
GR-5	0.47	0.46	1																							
GR-8	0.45	0.53	0.49	1																						
GR-9	0.39	0.38	0.4	0.4	1																					
Ashoka200F	0.49	0.58	0.42	0.54	0.38	1																				
AAUDR-1	0.36	0.49	0.37	0.59	0.35	0.5	1																			
DDR-22	0.41	0.49	0.29	0.5	0.33	0.53	0.59	1																		
Kalinga-III	0.36	0.39	0.29	0.42	0.29	0.51	0.44	0.56	1																	
Vandana	0.32	0.44	0.31	0.44	0.3	0.49	0.47	0.56	0.6	1																
Annanda	0.39	0.42	0.31	0.47	0.26	0.47	0.46	0.52	0.59	0.57	1															
GR-3	0.36	0.4	0.29	0.42	0.25	0.49	0.42	0.51	0.57	0.56	0.6	1														
GR-4	0.33	0.34	0.27	0.39	0.27	0.4	0.35	0.45	0.42	0.43	0.49	0.48	1													
GR-6	0.21	0.25	0.24	0.33	0.17	0.27	0.29	0.3	0.29	0.31	0.29	0.28	0.27	1												
GR-7	0.25	0.31	0.26	0.29	0.18	0.3	0.3	0.31	0.31	0.31	0.26	0.28	0.27	0.48	1											
GR-11	0.2	0.22	0.17	0.23	0.2	0.22	0.26	0.24	0.25	0.27	0.2	0.22	0.28	0.44	0.52	1										
GR-12	0.23	0.24	0.19	0.3	0.2	0.27	0.28	0.28	0.27	0.31	0.26	0.27	0.3	0.51	0.53	0.51	1									
Jaya	0.21	0.24	0.23	0.29	0.25	0.26	0.28	0.29	0.28	0.33	0.28	0.27	0.3	0.51	0.53	0.52	0.59	1								
Samba masuri	0.23	0.26	0.21	0.3	0.25	0.29	0.26	0.26	0.31	0.3	0.27	0.32	0.26	0.38	0.38	0.4	0.46	0.37	1							
IR-64	0.22	0.27	0.22	0.3	0.2	0.32	0.29	0.29	0.3	0.36	0.28	0.3	0.27	0.51	0.58	0.53	0.61	0.57	0.53	1						
Swama	0.26	0.29	0.25	0.3	0.21	0.29	0.29	0.33	0.33	0.33	0.3	0.31	0.29	0.47	0.48	0.39	0.54	0.55	0.4	0.57	1					
Lalat	0.23	0.27	0.2	0.33	0.18	0.32	0.29	0.33	0.35	0.35	0.32	0.36	0.28	0.46	0.47	0.32	0.49	0.47	0.43	0.58	0.59	1				
TN-1	0.21	0.28	0.21	0.31	0.18	0.28	0.29	0.28	0.29	0.36	0.27	0.29	0.26	0.47	0.43	0.36	0.53	0.52	0.34	0.53	0.57	0.59	1			
Tapswini	0.2	0.26	0.16	0.3	0.17	0.28	0.25	0.29	0.31	0.34	0.32	0.37	0.31	0.39	0.41	0.36	0.49	0.44	0.52	0.51	0.5	0.61	0.51	1		
Pusabasmati	0.21	0.27	0.21	0.32	0.2	0.31	0.31	0.33	0.32	0.36	0.29	0.34	0.29	0.41	0.39	0.28	0.45	0.44	0.34	0.48	0.53	0.59	0.52	0.53	1	
IET-18990	0.21	0.29	0.21	0.32	0.21	0.29	0.27	0.28	0.28	0.31	0.35	0.3	0.3	0.39	0.42	0.32	0.41	0.4	0.44	0.44	0.47	0.48	0.4	0.53	0.47	1

Table 5: Details of SSR markers used for molecular analysis

Sr. No	Name of the SSR marker	No. of Alleles	Range of allele length (bp)	Highest allele length observed in Genotype(s)	Allele frequency	Highest allele frequency observed in Genotype(s)	Expected Heterozygosity
1.	RM205	21	107-145	Sathi34-36	0.047 - 0.476	DDR-22 and GR-9	0.88
2.	RM213	24	109-134	SK-20	0.043 - 0.304	GR-12,Jaya,Samba Masuri,IR-64,Swama, and Tapaswini	0.80
3.	RM215	23	135-170	IET-18990	0.043 - 0.217	GR-5, AAUDR-1, DDR-22,Kalinga-III, and Vandana	0.87
4.	RM223	24	141-168	Lalat	0.38 - 0.269	GR-7,GR-12,Jaya, and Samba Masuri,IR-64,Basmati andIET-18990	0.84
5.	RM231	23	160 - 201	IR-64	0.043 - 0.173	GR-6, Jaya, TN-1, Tapaswini GR-7, GR-11, GR-12, IET-18990 GR-8, GR-9, Ashoka-200F and AAUDR-1	0.80
6.	RM234	26	129 - 165	IR-64	0.038-0.192	GR5,Ashoka-200F,Annadana,GR-3,GR-4	0.89
7.	RM242	19	184 -238	Vandana, GR-3	0.045 -0.181	Kalinga-III, Annanda, Swama, and Tapaswini.	0.90
8.	RM318	25	116 - 144	Sathi34-36	0.038 -0.346	GR-6, GR-7, GR-12, Jaya, Samba Masuri, lalat, TN-1, Pusa Basmati-1, IET-18990	0.68
9.	RM420	23	165 - 181	SambaMasuri	0.41 - 0.500	Sathi34-36,SK-20,GR-5,GR-9,AAUDR-1,GR-3,GR-11,Jaya,IR64, Swarna,lalat,IET-18990.	0.68
10.	RM3810	21	80 - 99	Pusa Basmati-1	0.045 -0.181	Ashok-200F, DDR-22, Kalinga-III, and Annanda.	0.83

Table 6: Similarity matrix for 26 rice genotypes based on SSR markers

	Sathi 34-36	sk-20	GR-5	GR-8	GR-9	Ashoka-200f	AAUDR-1	DDR-22	Kalinga-III	Vandana	Annanda	GR-3	GR-4
Sathi34-36	1												
sk-20	0.25	1											
GR-5	0.154	0.143	1										
GR-8	0.067	0.133	0.063	1									
GR-9	0.067	0.133	0.133	0.125	1								
Ashoka-200f	0	0.063	0.063	0.2	0.286	1							
AAUDR-1	0.067	0.063	0.214	0.2	0.385	0.125	1						
DDR-22	0.063	0.059	0.059	0	0	0.118	0.118	1					
Kalinga-III	0	0	0.063	0.059	0	0.125	0.059	0.188	1				
Vandana	0	0	0.214	0	0.059	0	0.2	0.056	0.125	1			
Annanda	0	0	0.063	0.059	0	0.059	0.059	0.118	0.059	0.125	1		
GR-3	0.071	0.067	0.143	0	0.063	0.063	0.063	0.059	0	0.063	0.308	1	
GR-4	0	0	0.083	0	0	0.077	0	0.071	0	0.077	0.4	0.444	1
GR-6	0	0	0	0	0	0	0	0.063	0	0	0	0	0
GR-7	0	0	0	0	0	0	0	0.059	0	0	0	0	0
GR-11	0.067	0.063	0.063	0	0.059	0	0.059	0	0	0	0	0.063	0
GR-12	0	0	0	0	0	0	0	0	0	0	0	0	0
Jaya	0.063	0.059	0.059	0	0.056	0	0.056	0	0	0	0	0.059	0
sambhamasuri	0	0	0	0	0	0	0	0	0	0	0	0	0
IR-64	0.063	0.059	0.059	0	0.056	0.056	0.056	0	0	0	0	0.059	0
Swarna	0.063	0.059	0.125	0	0.118	0	0.118	0	0.056	0.056	0.056	0.059	0
Lalat	0.063	0.059	0.125	0	0.118	0	0.118	0	0	0.056	0	0.059	0
TN-1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tapswini	0	0	0	0	0	0	0	0	0.056	0	0.056	0	0
Pusabasmati	0	0	0	0	0	0	0	0	0	0	0	0	0
IET-18990	0.063	0.059	0.059	0	0.056	0	0.056	0	0	0	0	0.059	0

	GR-6	GR-7	GR-11	GR-12	Jaya	Sambhamasuri	IR-64	Swarna	Lalat	TN-1	Tapswini	Pusa basmati	IET-18990
Sathi34-36													
sk-20													
GR-5													
GR-8													
GR-9													
Ashoka-200f													
AAUDR-1													
DDR-22													
Kalinga-III													
Vandana													
Annanda													
GR-3													
GR-4													
GR-6	1												
GR-7	0.25	1											
GR-11	0.067	0.214	1										
GR-12	0.067	0.214	0.2	1									
Jaya	0.133	0.286	0.188	0.267	1								
sambhamasuri	0.067	0.133	0.125	0.385	0.357	1							
IR-64	0	0.059	0.188	0.267	0.25	0.267	1						
Swarna	0	0	0.188	0.056	0.111	0.056	0.176	1					
Lalat	0.063	0.125	0.056	0.056	0.111	0.118	0.111	0.176	1				
TN-1	0.143	0.133	0	0.059	0.118	0.059	0	0.056	0.188	1			
Tapswini	0.063	0	0.056	0.056	0.111	0.056	0.053	0.176	0.053	0.583	1		
Pusabasmati	0.067	0.214	0	0.125	0.118	0.125	0.056	0	0.118	0.286	0.118	1	
IET-18990	0.063	0.286	0.118	0.188	0.25	0.118	0.111	0.053	0.176	0.267	0.111	0.267	1

highest allele frequency (0.500) was recorded by the marker RM 420 for varieties Sathi 34-36, SK-20, GR-5, GR-9, AAUDR-1, GR-3, GR-11, Jaya, IR-64, Swarna, Lalat and IET-18990. The expected heterozygosity amongst 26 rice genotypes was observed in the range of 0.68 to 0.90, where in the marker RM 242 revealed the highest value of 0.90. It was observed that when 26 rice genotypes were analysed at molecular level using ten markers, Sathi 34-36 and Lalat amplified highest allele length, maximum number of times. Similarly, for allele frequency, varieties Jaya and GR-12 appeared maximum number of occasions (Table 5). Dendrogram generated for 10

SSR markers against 26 rice genotypes formed two major clusters 'A' and 'B' (Fig 2). Cluster 'A' comprised mostly upland cultivars and a few lowland cultivars, whereas cluster 'B' consisted of mostly transplanted cultivars. Cluster 'A' is further divided into two sub-clusters 'A1' and 'A2'. Sub-cluster 'A1' comprised Sathi 34-36, SK-20, GR-8, GR-9, AAUDR-1 and Ashoka -200F, while sub-cluster 'A2' consisted of GR-5, Vandana, Annanda, GR-3, GR-4, DDR-22 and Kalinga-III. Sub cluster 'A1' included all upland rice varieties of Gujarat, except GR-5, whereas, sub-cluster 'A2' was a mixed group of upland and lowland varieties. Cluster 'B' has two sub-clusters 'B1'

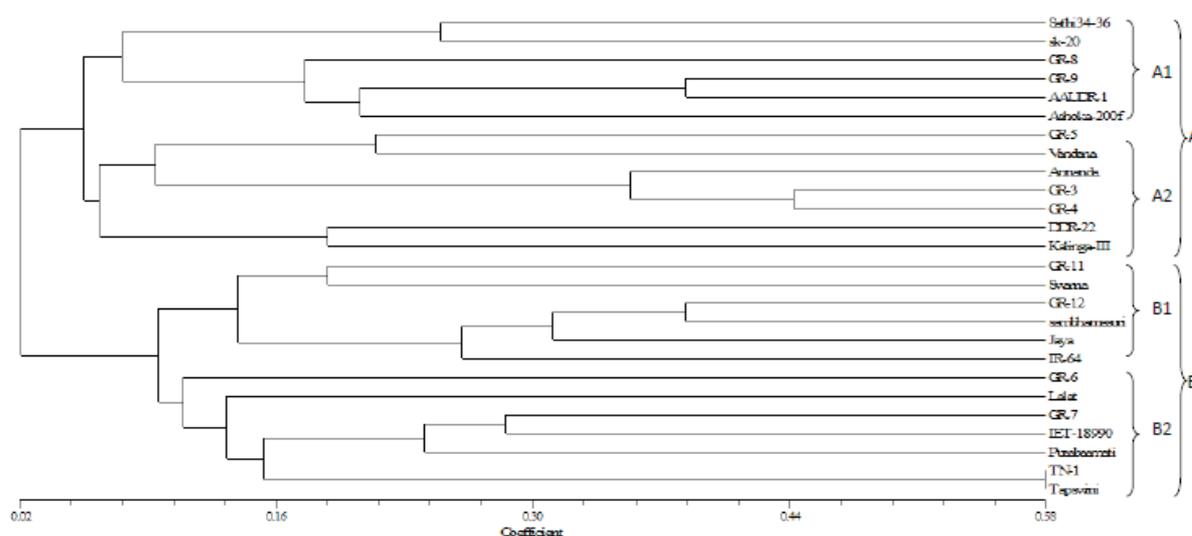


Figure 2: Dendrogram of genetic relationship among rice genotypes based on SSR markers.

and 'B2'. Sub-cluster 'B1' comprised GR-11, Swarna, GR-12, Sambhamasuri, Jaya, IR-64, whereas, sub-cluster 'B2' consisted of GR-6, Lalat, GR-7, IET-18990, Pusha Basmati-1, TN-1 and Tapaswini. The present work corroborates with the work of Noorzuraini *et al.*, (2013) where diversity assessment of 80 rice varieties were carried out through 119 SSR markers. Similarly, earlier studies on genetic diversity in rice also observed higher PIC values among various rice backgrounds such as cultivars, landraces and wild relatives (Ram *et al.*, 2007; Ravi *et al.*, 2003). These findings indicated high genetic diversity due to differences in origin, ecotype and speciation (Ram *et al.*, 2007). Moreover, SSR markers may exhibit high PIC values because of codominant expression, multiallelism (Ferreira and Grattapaglia 1998; Ram *et al.*, 2007) and mostly monolocus (Gracia *et al.*, 2004).

The similarity coefficient (Table 6) was found minimum between Tapaswini and IR-64 and Tapaswini and Lalat. whereas, Samba Masuri and GR-12 showed maximum similarity index (0.385). The RAPD analysis also showed similar pattern between Lalat and Tapaswini.

The advent and application of molecular analysis to population genetic structures provides extremely useful information that will undoubtedly prove invaluable to future decision making processes involved in the management and preservation of germplasm and genetic resources.

REFERENCES

- Ahmadikhah, A., Karlov, G. I., Nematzadeh, Gh. and Bezdi, K. G. 2007. Inheritance of the fertility restoration and genotyping of rice lines at the restoring fertility (*Rf*) loci using molecular markers. *Int. J. Pl. Prod.* **1**: 13-21.
- Anderson, J. A., Churchill, G. A., Autrique, J. E., Tanksley, S. D. and Sorrells, M. E. 1993. Optimizing parental selection for genetic linkage maps. *Genome*. **36**:181-186.
- Balakrishna, B. and Satyanarayana, P. V. 2013. Genetics of brown planthopper (*nilaparvata lugensta* L.) Resistance in elite donors of rice (*oryza sativa* L.). *The Bioscan*. **8(4)**: 1413-1416.
- Dhanwani, R. K., Sarawgi, A. K., Solanki A. and Tiwari, J. K. 2013. Genetic variability analysis for various yield attributing and quality traits in rice (*Oryza sativa* L.). *The Bioscan*. **8(4)**: 1403-1407.
- Davla, D., Sashidharan, N., Macwana, S., Chakraborty, S., Trivedi, R., Ravikairan, R. and Shah, G. 2013. Molecular characterization of rice (*Oryza sativa* L.) genotypes for salt tolerance using microsatellite markers. *The Bioscan*. **8(2)**: 499-502.
- David, C. C. and Otsuka, K. 1994. Differential impact of modern rice varieties in Asia: an overview. In: C.C. David and K. Otsuka (eds.). *Modern rice technology and income distribution in Asia* (475p). London: Lynne Rienner Publisher, Inc. pp.11-22.
- Degenkolbe, T., Do, P. T., Kopka, J., Zuther, E., Hincha, D. K. and Kohl, K. I. 2013. Identification of drought tolerance markers in a diverse population of rice cultivars by expression and metabolite profiling. *PLOS One*. **8(5)**: 1-14.
- Ferreira, M. E. and Grattapaglia, D. 1998. Introducao ao Uso de Marcadores. *Molecular em Analise Genetica*, 3rd Edition. Embrapa: Brasilia. p. 220.
- Gracia, A. A., Benhimol, L. L., Antonica, M. M., Geraldia, I. O. and Deuza, A. P. 2004. Comparison of RAPD, RFLP, AFLP and SSR marker for diversity studies in tropical maize inbred lines. *Euphytica*. **108**: 53-63.
- Islam, M. S., Ali, M. A., Guswani, P., Ullah, S. M. S., Hossain, M. M., Miah, M. F. and Prodhan, S. H. 2013. Assessment of genetic diversity among moderately drought tolerant landraces of rice using RAPD markers. *J. Bio. Biotech.* **2(3)**: 207-213.
- Noorzuraini, A. R., Borromeo, T. H., Nestor, N. C., Diaz, N. C., Diaz, G. M. and Arvind, K. 2013. Diversity assessment of Malaysian rice germplasm accessions for drought tolerant grain yield QTLs. *J. Trop. Agri. Food Sci.* **4(1)**: 27-40.
- Ogunbayo, S. A., Ojo, D. K., Guei, R. G., Oyelakin, O. O. and Sanni, K. A. 2005. Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques. *Afr. J Biotech.* **4(11)**: 1234-1244.
- Ram, S. G., Thiruvengadam, V. and Vinod, K. K. 2007. Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellites markers. *J. App. Gen.* **48(4)**: 337-345.
- Ravi, M., Geethanjali, S., Sameeyafarheen, F. and Meheswaran, M. 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR. *Euphytica*. **133**: 243-252.

Reddy, E. S., Verma, S. K., Xalxo, S. M., Saxena, R. R. and Verulkar, S. B. 2013. Identification of molecular markers for root length in rice (*Oryza sativa* L.). *The Bioscan*. **8(4)**: 1511-1514.

Smith, B. D. 1998. The emergence of Agriculture. *Scientific American Library, a division of HPHP, New York*.

Souza, F. F., Caixeta, E. T., Ferrao, L. F. V., Pena, G. F., Sakiyama, N.

S., Zambolim, E. M., Zambolium, L. and Cruz, C. D. 2013. Molecular diversity in *Coffea canephora* germplasm conserved and cultivated in Brazil. *Crop breed. Appl. Biotechnol.* **13**: 221-227.

Williams, K., Kubelik, A., Livak, K., Rafalski, J. and Tingey, V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.* **18**: 6531-6535.

