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GENETICS AND PLANT BREEDING

Genetic Diversity Analysis in Rice (*Oryza sativa* L.) Accessions using SSR Markers

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ABSTRACT

Sixty-five rice accessions were analyzed to evaluated the genetic polymorphism and identification of diverse parents using simple sequences repeat (SSR) markers. These accessions showed significant phenotypic variation for all the characters studied. A total of 52 alleles were detected by 19 polymorphic markers showing highly polymorphic across all accessions with an average of 2.7 alleles per polymorphic marker. The marker RM-84 and RM- 481 produced maximum 4 alleles. The PIC value ranged from 0.032 to 0.588 and marker RM231 was found to be the most appropriate marker to discriminate among the rice genotypes owing to the highest PIC value of 0.588. The cluster analysis showed that these accessions grouped in to nine clusters in which cluster IB-1a had maximum thirty-one genotypes followed by cluster IB-1b and cluster V. While highest dissimilarity coefficient value was observed between the cultivar LC-4 and IR-82635-B-B-47- and between OR-1946-2-1 and UPLRI-7 showing highly diverse genotypes. These accessions were showing wide genetic divergence among the constituent in it and may be directly utilized in hybridization programme for improvement of yield related traits.

Highlights

- 52 alleles were detected with an average of 2.7 alleles per polymorphic marker.
- PIC value ranged from 0.032 to 0.588, indicates that markers were highly informative and capable of distinguishing between genotypes.
- Cultivar LC-4 and IR-82635-B-B-47-1 (0.0429) identified as highly diverse genotypes on the basis of dissimilarity coefficient.

Keywords: *Oryza sativa* accessions, Molecular diversity, SSR, PIC

Rice an important food for about half of the world's population and 90% of it is being produced and consumed in Asia (Rao *et al.* 2016) and share maximum in grain production. India is one of the centers for rice diversity (Singh *et al.* 2016). The rice accessions are a rich reservoir of useful genes that rice breeder can harness for rice improvement programme and the genetic variability exists among rice accessions leaving a wide scope for crop improvements (Singh *et al.* 2015). Genetic diversity is necessary for any crop improvement program as it helps in analyzing and establishing genetic relationship in accessions collection, its monitoring, identification of diverse parental combinations to create segregating progenies with high genetic variability and to obtain potential recombinations for further selection and introgression of desirable genes from these diverse accessions (Ramadan *et al.* 2015; Thompson *et al.* 1998 and Islam *et al.* 2012). Since been a long time a major goal in evolutionary biology is to Characterize and quantify the genetic diversity. Determination of genetic diversity can be

done by assessing morphological or molecular data. The use of advanced molecular technologies is one possible approach to understand their diversity. Evaluation of genetic diversity using DNA marker technology is non-destructive, not affected by environmental factors, requires small number of samples, and does not require large experimental setup and equipments for measuring physiological parameters (Kanawapee *et al.* 2011).

Simple sequence repeat (SSR) is an important tool for genetic variation identification of accessions (Sajib *et al.* 2012, Ma *et al.* 2011). SSR marker are highly informative, mostly monolocus, codominant, easily analyzed and cost effective (Gracia *et al.* 2004) and able to detect high level of allelic diversity (Ni *et al.* 2002), thus being widely applied in genetic diversity analysis, molecular map construction and gene mapping (Zhang *et al.* 2007, Ma *et al.* 2011), and analysis of germplasm diversity (Zhou *et al.* 2003, Jin *et al.* 2010, Ma *et al.* 2011). SSR markers even in less number can give a better genetic diversity spectrum due to their multi-allelic and highly polymorphic nature (Singh *et al.* 2016). Reports suggest that genetic diversity in crop varieties released over the years fluctuates in successive time periods (Upadhyay *et al.* 2012). Over the last few centuries, rice has faced diversity loss (Choudhary *et al.* 2013) especially, after the green revolution due to replacement of native varieties with high yielding varieties (Heal *et al.* 2004).

Therefore the present study was undertaken with the aim to assess the trend in genetic diversity in sixty five accessions of rice using SSR markers. The generated information will enable maximized selection of diverse parents and selecting appropriate parental genotypes in breeding programme.

MATERIALS AND METHODS

The experiment was carried out during *kharif* season 2015 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The experimental seed material comprises of sixty five rice accessions provided by DBT Networking Project (Table 1). The nursery was sown on on 12th June, 2015 on uniform raised beds applied with a fertilizer dose of 1.0 Kg N, 1.0 Kg P_2O_5 and 0.5 Kg K_2O per 50 m^2 area. 21 days old seedling was transplanted in a randomized block design (RBD) with three replications by maintaining row to row and plant to plant spacing 20 × 15 cm, respectively.

Table 1: List of rice accessions used under study

Sl. No.	Accessions	Sl. No.	Accessions	Sl. No.	Accessions
1	OR 1946-2-1	2	IR 83142-76	3	Vandana
4	IR 82635-B-B-47-1	5	IR 82589-B-B-7-2	6	IR 82635-B-B-23-1
7	CRR 660-2	8	CRR 428-237-1-3-1	9	Rewa 1208-15
10	IR 83399-B-B-52-1	11	PAU 3832-79-4-3-1	12	IR 83182-6-4
13	IR 78755-190-B-1-3	14	IR 82635-B-B-25-4	15	RP 5345-9-6-3
16	RRF-48	17	IR 55423-01	18	CB 10-504
19	GK 5022	20	Anjali	21	IR 10L-105
22	B 11576F-MR-18-2	23	CR 3631-1-3	24	BAU 411-05
25	IR 82921-B-B-1	26	BD 104	27	IR 77298-14-1-2-13
28	CR 422-63-51-B-2-1-1-1-B	29	IR 1718-59-1-2-3	30	IR 82635-B-B-145-1
31	NDR 1140	32	CR 3633-1-2	33	IR 368B-TB-25-MP-2
34	UPLRI – 7	35	IR 87694-28-7-2-1	36	RP 5330-63-5-2-1-B
37	MGD 1206	38	IR83867-B-B-250-CRA-1-1	39	IR 83926-B-B-71-4
40	IR 60080-46A	41	BD 108	42	BVS 1
43	BVD 111	44	BVD 203	45	BAU 389-02
46	BAU/IRRI 497	47	LC -1	48	LC -2
49	LC -3	50	LC -4	51	LC -5
52	LC – 6	53	LC – 7	54	LC – 8
55	LC – 9	56	LC – 10	57	LC – 11
58	LC – 12	59	LC – 13	60	LC – 14
61	LC – 15	62	LC – 16	63	LC – 17
64	LC – 18	65	LC – 19		

Morphological assessment

Observations were recorded on eleven quantitative traits *viz.*, days to 50% flowering (DF), days to maturity (DM), number of effective tillers per plant (ET), plant height (PH), panicle length (PH), panicle weight (PW), filled grains per panicle (FG), total grains per panicle (TG), spikelet fertility percent (SFP), test weight (TW) and grain yield per plant (GY).

Genomic DNA extraction

Young leaves of 15-20 days old seedlings from sixty five rice genotypes were clipped and stored in ice-box to carry it to the lab which is then stored in -80^{0}C till DNA extraction. Genomic DNA was then extracted using CTAB method (Doyle and Doyle 1987). DNA samples were diluted to 10 ng/µl. The DNA was quantified spectrophotometrically (PerkinElmer, Singapore) by measuring A260/A280 and DNA quality was checked by electrophoresis in 0.8% agarose gel.

SSR Markers and PCR amplification

A total of twenty rice SSR markers were used for molecular diversity (Table 2). The PCR amplification was carried out in 15µl of reaction mixture containing 30 ng genomic DNA, 1.5 mM PCR buffer (MBI Fermentas, USA), 400 µMdNTPs (MBI Fermentas), 1 U Taq DNA polymerase (MBI Fermentas) and 0.4 µM primer using a thermal cycler (Master cycler gradient, Eppendorf). Thermal cycling program involved an initial denaturation at 94° for 45 sec, annealing at 2° bellow Tm of respective primers for 30 sec, primer extension at 72° for 30 sec, followed by a final extension at 72° for 7 min. Electrophoresis separation and visualization of amplified products. The amplified PCR products along with a 50 bp DNA marker ladder (MBI Fermentas) were size fractioned by electrophoresis in 2.5% agarose gel prepared in TAE buffer and visualized by staining with ethidium bromide (0.5 µg/ml) in a gel documentation system (BIO-RAD, USA). The reproducibility of amplification products was compared twice for each primer.

SSR data analysis

Standardization of quantitative data

The effects of different scales of measurement for different quantitative traits were minimized by standardizing the data for each trait separately prior to cluster analysis. Standardization was done by dividing the deviation of mean for a line from the mean for sixty five lines with the standard deviation for the given trait; the STAND module of NTSYS (Rohlf 1997) software was used to furnish the same.

Genetic dissimilarity and Cluster analysis based on UPGMA

The binary data matrix generated by polymorphic SSR markers were subjected to further analysis using NTSYS-pc version 2.11W (Rohlf 1997). The SIMQUAL program was used to calculate the Jackard' dissimilarity coefficient. The dissimilarity matrix was used as an input for analysis of clusters. UPGMA-based clustering was done using SAHN module of NTSYSpc for dendrogram construction. In Unweighted pair-group average (UPGMA) clusters are joined based on the average distance between all members in the two groups.

Polymorphic information content (PIC) and Principal component analysis (PCA)

PIC for SSR markers was calculated as per the formula:

$$PIC = 1 - P_{ij}^2$$

where, PIC*i* is the polymorphic information content of a marker *i* and the summation extends over *n* patterns. PCA was also done to check the result of UPGMA base clustering using EIGEN module of NTSYSpc. In principal component analysis (PCA), the total variance of original characters is divided into a limited number of uncorrelated new variables known as principal components (PCs). The first step in PCA is to calculate eigen values, which define the amount of total variation that is displayed on PC axes. The first PC summarizes most of the variability not summarized by, and uncorrelated with, the first PC, and so on. PCs were used for 2-dimentional (2-D) and 3-dimentional (3-D) plotting, respectively, against each other using module PROJ and MXPLOT of NTSYSpc.

RESULTS AND DISCUSSION

Polymorphism and marker efficiency

Sixty five rice genotypes were subjected to SSR

Table 2: Mean performance of sixty five rice accessions for various characters

Sl. No.	Character	Days to 50% Flowering	Days to Maturity	No. of Tillers	Plant Height	Panicle Length (cm)	Panicle Weight (gm)	Filled Grains Per Panicle	Grains Per Panicle	Spikelet Fertility (%)	Test Weight (gm)	Grain Yield Per Plant (gm)
1	OR 1946-2-1	99.67	135.67	12.93	107.69	28.43	3.78	151.67	180.20	83.54	23.83	32.51
2	IR 83142-76	130.67	164.33	7.73	188.54	26.03	4.98	193.93	212.93	91.12	23.88	29.36
3	Vandana	102.67	137.00	6.27	126.15	26.19	2.98	104.33	135.40	76.82	24.97	16.66
4	IR 82635-B-B-47-1	131.33	165.33	14.00	172.51	27.28	3.01	147.87	165.27	89.40	17.72	19.97
5	IR 82589-B-B-7-2	127.33	156.67	8.13	85.79	22.28	2.22	115.33	131.91	86.95	18.83	14.53
6	IR 82635-B-B-23-1	155.33	180.33	10.33	177.21	25.47	2.78	80.73	112.40	72.00	24.89	16.24
7	CRR 660-2	91.00	121.00	9.67	110.89	22.98	2.90	122.33	133.27	88.67	21.94	18.41
8	CRR 428-237-1-3-1	83.67	115.67	11.53	110.48	20.28	2.29	102.47	113.87	89.83	19.70	20.36
9	Rewa 1208-15	130.33	164.33	10.73	170.48	22.77	2.46	152.67	191.53	78.77	15.63	18.07
10	IR 83399-B-B-52-1	126.33	159.67	8.60	91.23	27.43	3.94	139.07	166.80	84.30	25.41	25.76
11	PAU 3832-79-4-3-1	110.67	140.33	9.33	81.14	23.24	2.22	113.00	136.00	79.00	16.75	20.36
12	IR 83182-6-4	126.00	161.00	10.07	146.77	26.87	2.91	123.27	143.33	90.05	21.49	26.59
13	IR 78755-190-B-1-3	89.67	120.00	9.27	131.28	22.36	2.30	87.27	90.87	93.12	24.69	15.43
14	IR 82635-B-B-25-4	84.33	115.00	9.73	84.73	23.50	1.45	61.00	83.73	71.03	20.21	16.95
15	RP 5345-9-6-3	87.67	116.67	15.00	120.05	20.95	2.30	104.53	132.80	79.42	19.85	22.21
16	R RF-48	96.67	125.00	11.07	91.01	23.42	1.85	81.13	98.93	84.49	19.09	13.48
17	IR 55423-01	95.00	128.00	8.00	112.93	26.59	3.41	143.73	169.40	78.93	22.39	21.98
18	CB 10-504	90.67	122.67	11.60	97.63	24.11	2.20	102.80	124.07	82.68	20.12	19.33
19	GK 5022	111.67	140.67	7.47	125.45	27.25	5.77	198.00	220.73	82.74	28.68	32.36
20	Anjali	107.33	138.33	9.60	123.09	31.49	2.46	137.80	177.07	77.69	16.72	16.86
21	IR 10L-105	97.67	137.00	11.13	108.24	27.01	2.45	86.53	118.40	72.93	24.91	23.15
22	B 11576F-MR-18-2	138.00	163.33	10.27	137.22	27.57	2.15	177.60	234.20	84.76	10.02	16.04
23	CR 3631-1-3	144.33	178.00	10.47	177.50	23.48	3.25	122.80	171.67	71.65	20.19	24.42
24	BAU 411-05	83.67	115.67	11.93	92.03	23.50	1.82	75.73	104.00	72.46	23.71	16.52
25	IR 82921-B-B-1	95.00	122.33	8.27	142.71	24.95	5.41	210.07	251.33	89.20	22.14	30.81
26	BD 104	85.00	119.33	10.93	136.74	25.68	3.51	102.60	119.33	86.12	32.30	29.61
27	IR 77298-14-1-2-13	131.33	164.33	7.73	123.29	25.46	3.29	140.13	169.80	82.51	21.03	23.25
28	CR 422-63-51-B-2-1-1-1-B	96.00	131.00	9.53	109.72	26.09	2.83	97.07	111.80	87.15	28.24	21.64
29	IR 1718-59-1-2-3	95.00	130.33	6.60	116.47	25.05	3.18	126.00	167.40	70.44	22.39	22.12
30	IR 82635-B-B-145-1	112.33	142.00	11.80	117.81	22.85	3.14	166.33	183.33	90.26	17.74	30.35
31	NDR 1140	103.33	137.00	9.80	98.11	23.41	3.00	199.87	226.13	85.74	14.89	22.25
32	CR 3633-1-2	101.00	137.67	7.00	109.51	27.60	3.50	105.93	175.60	60.38	26.41	17.60
33	IR 368B-TB-25-MP-2	99.33	137.00	7.47	99.71	24.35	2.12	111.40	131.53	82.90	20.86	18.33
34	UPLRI - 7	121.67	157.67	14.20	136.75	23.15	2.44	87.73	107.67	81.73	24.46	22.84
35	IR 87694-28-7-2-1	123.33	157.00	7.67	90.20	21.97	2.82	135.67	147.47	91.83	20.57	19.11
36	RP 5330-63-5-2-1-B	91.00	122.33	14.87	133.97	26.57	2.93	114.27	122.13	93.63	24.71	33.90
37	MGD 1206	82.33	111.67	10.00	89.89	23.47	1.98	90.40	114.67	78.55	21.84	18.50
38	IR 83867-B-B-250-CRA-1-1	95.33	123.67	10.07	95.23	26.26	3.02	140.60	203.47	69.26	19.01	24.95
39	IR 83926-B-B-71-4	87.33	116.00	10.40	63.42	15.79	0.91	43.93	52.00	84.30	19.84	5.22
40	IR 60080-46A	82.33	114.67	13.00	91.07	24.78	2.32	93.67	107.20	87.44	22.12	23.87
41	BD 108	125.33	157.00	10.80	128.57	22.13	3.20	163.47	200.20	81.77	17.37	26.47

42	BVS 1	92.00	124.67	7.40	112.69	25.07	3.25	125.80	157.67	79.82	21.75	21.66
43	BVD 111	132.67	167.00	10.47	166.08	28.88	2.81	107.53	142.53	74.86	21.29	27.92
44	BVD 203	100.33	132.67	6.27	135.36	28.04	3.50	137.87	180.80	77.93	23.03	20.89
45	BAU 389-02	93.00	124.67	8.93	125.00	28.43	3.96	146.13	193.67	75.67	24.45	30.50
46	BAU/IRRI 497	136.67	167.67	7.60	139.69	30.25	2.78	152.87	187.13	78.55	17.26	17.93
47	LC - 1	132.67	165.33	10.20	156.79	24.86	2.26	155.73	220.13	70.84	12.64	21.47
48	LC - 2	102.67	138.67	9.13	122.88	24.81	3.13	114.13	137.73	80.74	26.46	24.08
49	LC - 3	94.67	130.00	8.73	105.38	28.72	3.01	108.67	136.33	79.66	23.41	21.66
50	LC - 4	104.67	138.33	8.07	100.20	25.15	3.09	138.20	149.00	92.71	21.63	18.93
51	LC - 5	103.00	141.33	9.13	149.80	27.11	4.88	186.93	199.80	91.73	25.13	33.09
52	LC - 6	101.67	139.00	9.33	113.02	27.67	4.01	148.87	187.47	79.73	25.93	23.95
53	LC - 7	97.33	137.00	6.87	109.09	26.77	3.26	113.47	179.07	63.18	23.04	19.70
54	LC - 8	114.67	141.00	9.27	112.67	26.15	3.50	132.53	142.13	90.83	25.77	24.56
55	LC - 9	96.00	137.00	10.67	95.29	23.28	1.59	73.07	101.80	71.71	19.78	13.27
56	LC - 10	109.33	142.33	8.07	155.49	33.38	4.32	142.87	166.93	89.07	29.72	28.63
57	LC - 11	99.67	134.67	9.33	117.81	27.87	4.11	162.13	187.73	86.06	25.30	25.98
58	LC - 12	83.00	116.00	12.60	95.16	23.51	1.85	73.47	103.13	66.72	21.32	21.69
59	LC - 13	96.00	127.67	8.40	117.84	26.20	2.84	129.73	170.33	76.35	20.97	22.40
60	LC - 14	101.67	131.67	9.87	147.23	27.87	4.31	168.00	182.53	91.91	23.93	31.00
61	LC - 15	148.33	176.00	9.60	119.09	21.37	2.88	118.87	155.40	71.46	20.54	21.50
62	LC - 16	96.67	131.00	7.47	115.12	26.01	3.62	151.47	199.87	75.59	23.00	22.22
63	LC - 17	98.67	137.00	7.67	105.34	26.51	3.17	132.87	157.33	84.50	21.93	17.62
64	LC - 18	96.67	128.67	8.13	111.93	28.29	3.65	160.30	187.57	85.47	24.09	23.91

marker assay to assess the molecular diversity. Out of twenty primers used, 19 produced reproducible and polymorphic pattern while one primer (RM39) was monomorphic. The 19 polymorphic primers yielded a total of 52 fragments (amplified products). The size of fragments varied from 50bp (marker RM84) to 290bp (marker RM424). Maximum fragments were produced by primers RM84 and RM481 which yielded four fragments each and an average of 2.7 fragments was produced per primer which showed polymorphic amplification. Gel image showing SSR banding profile obtained by primer RM17 is presented in Fig. 1.

The polymorphic information content (PIC) was employed for each locus to assess the information of each marker and its discriminatory ability. PIC value refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency; thus, it provides an estimate of the discriminating power of the marker (Nagy *et al.* 2012). The PIC value is an evidence of allele diversity and frequency among varieties. The PIC value of SSR markers ranged from 0.032 to 0.588 with a mean PIC of 0.366. Markers RM231, RM17, RM481 and RM174 were the most informative primers on the basis of highest PIC of 0.588, 0.585, 0.497 and 0.467 respectively. SSR marker RM270 showed least PIC value of 0.032 (Table 3).

Dendrogram analysis

A dendrogram (Fig. 2) based on Jackard's dissimilarity coefficient was constructed using UPGMA. The genetic divergence was studied based on D^2 statistics. The sixty five rice accessions were grouped into two main clusters (Table 4) i.e. cluster I and cluster II with dissimilarity coefficient (0.15). Cluster I was further sub-divided into two minor sub-groups IA and IB with dissimilarity coefficient (0.28). Cluster IA and IB were further sub-divided into two subgroups i.e. IA-1 and IA-2 (0.31) and IB-1 and IB-2 (0.32) respectively. The second main cluster was also sub-divided into two minor subgroups i.e. IIA and IIB with dissimilarity coefficient 0.16. This indicated presence of considerable diversity in the accessions studied. The most diverse genotype is therefore, important in order to select desirable genotypes for utilizing in breeding programmes.

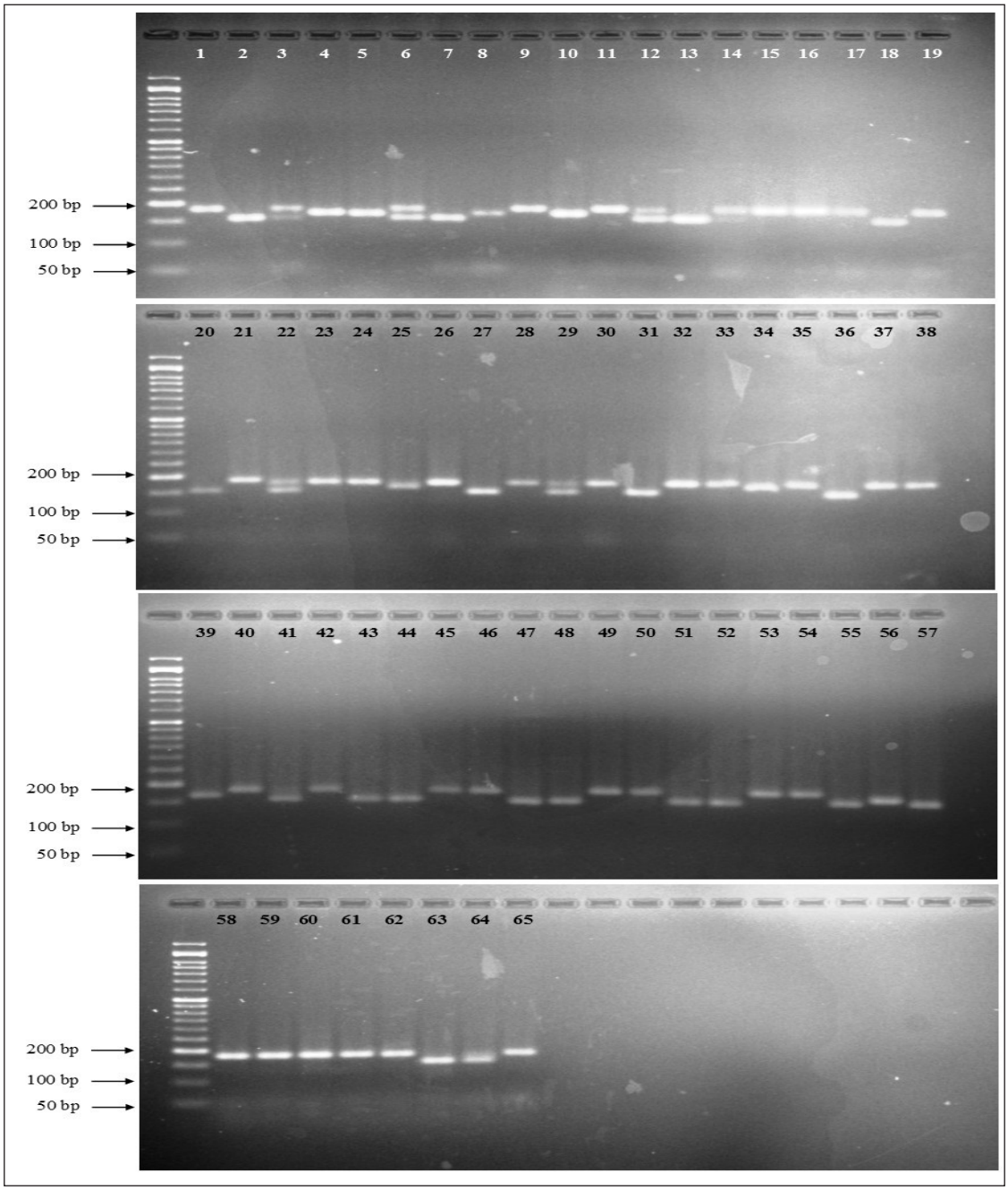

Fig. 1: SSR banding profile obtained by marker RM17. Lane 1-65 represents rice cultivar used in the present study; M = 50bp DNA size markers

On the basis of dendrogram, the highest similarity was observed between Rewa 1208-15 and PAU 3832-79-4-3-1 followed by LC-15 and LC-16, BAU 411-05 and IR 82635-B-B-145-1 and BVD 111 and LC-11. The most diverse cultivar was IR 82635-B-B-47-1 and OR 1946-2-1. These accessions were grouped into nine clusters. Cluster indicated that 31 germplasm out of sixty-five belong to the cluster IB-1a followed by cluster IB-1b which has 24 accessions and cluster IB-1c with 3 accessions. Clusters IA-1, IA-2, IIA-1, IIA-2 and IIB were monogenic in nature containing single accession each i.e. IR 82635-B-B-47-1, LC-10, IR 83926-B-B-71-4 and OR 1946-2-1, respectively.

Jackard dissimilarity coefficient

The dissimilarity coefficient varies from one to zero, close to one shows high similarity while close to zero shows high dissimilarity. The average of dissimilarity coefficient varies from 0.77 to 0.51. The total average of dissimilarity coefficient of all

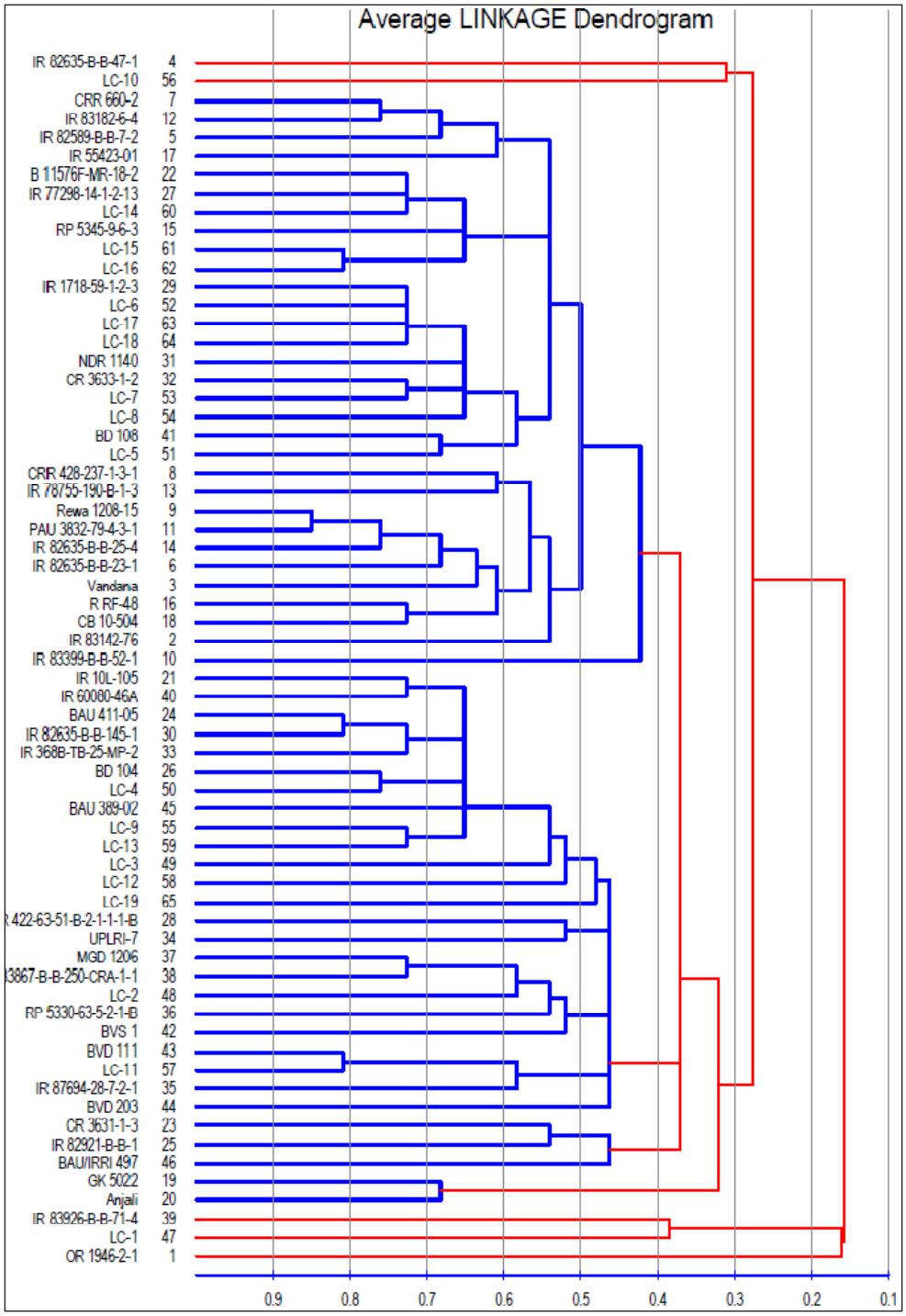

Fig. 2: Jackard IJ Distance

sixty five cultivar is 0.61. the dissimilarity coefficient varied from the largest value 0.94 between the cultivar LC-4 and IR 82635-B-B-47-1 followed by cultivar value 0.93 between the cultivar OR 1946-2-1 and UPLRI-7 which shows high similarity between them and it may be expected that both of them may have arouse from the same parents. The lowest value 0.15 was found between REWA 1208-15 and PAU 3832-79-4-3-1 followed by 0.19 between the cultivar BAU 411-05 and IR 82635-B-B-145-1, LC-15 and LC-16, and BVD 111 and LC-11 showing that they are highly dissimilar from each other. Cultivar IR 82635-B-B-47-1 shows highest similarity with cultivar LC-4 (0.94) and highest dissimilarity with CRR 428-237-1-3-1 (0.58). LC-15 has highest similarity with IR 82635-B-B-47-1 (0.85) and

Table 3: Details of the SSR primers used in present study, Allele size (bp) and polymorphism information content (PIC)

Sl. No.	SSR Primer	Sequence	Chr. No.	Tm(ºC)	No. of Alleles amplified	Approx. size of amplified product (bp)	PIC
1	RM1 F	GCGAAAACACAATGCAAAAA	1	54.7	2	80-110	0.294
	RM1 R	GCGTTGGTTGGACCTGAC					
2	RM17 F	TGCCCTGTTATTTTCTTCTCTC	-	58.55	3	160-190	0.585
	RM17 R	GGTGATCCTTTCCCATTTCA					
3	RM424 F	TTTGTGGCTCACCAGTTGAG	2	55.5	3	230-290	0.458
	RM424 R	TGGCGCATTCATGTCATC					
4	RM16874 F	TAGCAAGCTTGGAGAAGTGATGG	4	61.8	2	190-210	0.084
	RM16874 R	CAGAAGAAGTCAGCTCTATGCTTGG					
5	RM190 F	CTTTGTCTATCTCAAGACAC	6	54.5	3	135-180	0.382
	RM190 R	TTGCAGATGTTCTTCCTGATG					
6	RM481 F	TAGCTAGCCGATTGAATGGC	7	57.3	4	160-245	0.497
	RM481 R	CTCCACCTCCTATGTTGTTG					
7	RM434 F	GCCTCATCCCTCTAACCCTC	9	59.35	2	150-170	0.231
	RM434 R	CAAGAAAGATCAGTGCGTGG					
8	RM216 F	GCATGGCCGATGGTAAAG	10	55.65	3	110-145	0.418
	RM216 R	TGTATAAAACCACACGGCCA					
9	RM202 F	CAGATTGGAGATGAAGTCCTCC	11	57.6	2	150-190	0.281
	RM202 R	CCAGCAAGCATGTCAATGTA					
10	RM174 F	AGCGACGCCAAGACAAGTCGGG	2	65.8	3	210-250	0.467
	RM174 R	TCCACGTCGATCGACACGACGG					
11	RM 231 F	CCAGATTATTTCCTGAGGTC	3	55.6	3	175-205	0.588
	RM231 R	CACTTGCATAGTTCTGCATTG					
12	RM232 F	CCGGTATCCTTCGATATTGC	3	58.05	3	140-175	0.397
	RM232 R	CCGACTTTTCCTCCTGACG					
13	RM263 F	CCCAGGCTAGCTCATGAACC	2	60.4	2	160-195	0.364
	RM 263 R	GCTACGTTTGAGCTACCACG					
14	RM270 F	GGCCGTTGGTTCTAAAATC	12	57.95	2	125-140	0.032
	RM270 R	TGCGCAGTATCATCGGCGAG					
15	RM 39 F	GCCTCTCTCGTCTCCTTCCT	5	57.55	1	120 (monomorphic)	0.0
	RM39 R	AATTCAAACTGCGGTGGC					
16	RM335 F	GTACACACCCACATCGAGAAG	4	59.8	2	110-165	0.368
	RM335 R	GCTCTATGCGAGTATCCATGG					
17	RM528 F	GGCATCCAATTTTACCCCTC	6	57.3	3	180-220	0.447
	RM528 R	AAATGGAGCATGGAGGTCAC					
18	RM551 F	AGCCCAGACTAGCATGATTG	4	58.35	2	195-230	0.226
	RM551 R	GAAGGCGAGAAGGATCACAG					
19	RM84 F	TAAGGGTCCATCCACAAGATG	1	56.9	4	50-170	0.432
	RM84 R	TTGCAAATGCAGCTAGAGTAC					
20	RM87 F	CCTCTCCGATACACCGTATG	5	58.35	3	75-150	0.401
	RM87 R	GCGAAGGTACGAAAGGAAAG					

Table 4: Grouping of sixty-five rice accession into different clusters based on Jaccard's IJ coefficient

Cluster	Number of Genotypes	Name of the Genotypes
IA-1	1	IR 82635-B-B-47-1
IA-2	1	LC-10
IB-1a	31	CRR 660-2, IR 82589-B-B-7-2, IR 83182-6-4, RP 5345-9-6-3, IR 55423-01, B 11576F-MR-18-2, IR 77298-14-1-2-13, IR 1718-59-1-2-3, IR 83142-76, Vandana, IR 82635-B-B-23-1, CRR 428-237-1-3-1, Rewa 1208-15, IR 83399-B-B-52-1, PAU 3832-79-4-3-1, IR 78755-190-B-1-3, IR 82635-B-B-25-4, R RF-48, CB 10-504, NDR 1140, CR 3633-1-2, IR 60080-46A, LC – 5, LC – 6, LC – 7, LC – 8, LC-14, LC-15, LC-16, LC-17, LC-18
IB-1b	24	IR 10L-105, BAU 411-05, BD 104, CR 422-63-51-B-2-1-1-1-B, IR 82635-B-B-145-1, IR 368B-TB-25-MP-2, UPLRI – 7, RP 5330-63-5-2-1-B, MGD 1206, IR 83867-B-B-250-CRA-1-1, IR 87694-28-7-2-1, IR 60080-46A, BVS 1, BVD 111, BVD 203, BAU 389-02, LC-2, LC-3, LC-4, LC-9, LC-11, LC-12, LC-13, LC-19
IB-1c	3	CR 3631-1-3
		IR 82921-B-B-1
		BAU 389-02
IB-2	2	GK 5022
		Anjali
IIA-1	1	IR 83926-B-B-71-4
IIA-2	1	LC-1
IIB	1	OR 1946-2-1

dissimilarity with genotype LC-16 (0.19). Similarly, GK 5022 has highest similarity (0.88) with BAU/IRRI 497 and highest dissimilarity (0.32) with Anjali.

The level of polymorphism among rice genotypes was evaluated by calculating allelic number and PIC values for each of the nineteen polymorphic SSR markers. A total of 52 alleles were detected by 19 polymorphic markers across sixty five rice accessions with an average of 2.7 alleles per polymorphic marker. Among the polymorphic markers, 8 produced 2 alleles each, 9 markers had produced 3 alleles each and 2 of them produced a maximum of 4 alleles each. The amplicon size varied from 50 bp produced by RM84 to 290 bp produced by marker RM424. PIC value is a reflection of allele diversity and frequency among genotypes. The PIC value observed in the present investigation ranged from 0.032 to 0.588 with a mean PIC of 0.366; comparable to three previous estimates of microsatellite analysis in rice viz., 0.26 to 0.65 with an average of 0.47 (Singh *et al.* 2015), 0.28-0.50 with a mean of 0.45 (Umadevi *et al.* 2014) and 0.239 to 0.765 with an average of 0.508 (Hossain *et al.* 2012). The higher the PIC value of a locus, the higher the number of alleles detected. RM231 was found to be the most appropriate marker to discriminate among the rice genotypes owing to the highest PIC value of 0.588.

The dendrogram showed a total nine clusters on the basis of dissimilarity coefficient values. In the dendrogram cluster IB-1a had maximum thirty-one genotypes followed by cluster IB-1b and cluster IB-1c. Clusters IA-1, IA-2, IIA-1, IIA-2 and IIB have one germplasm each. On the basis of dendrogram the highest similarity observed between cultivar Rewa 1208-15 and PAU 3832-79-4-3-1 followed by LC-15 and LC-16, BAU 411-05 and IR 82635-B-B-145-1 and BVD 111 and LC-11. The most diverse cultivar was IR 82635-B-B-47-1 and OR 1946-2-1. Similar result was found also by Singh *et al.* (2015) where the accessions were grouped in two major groups and 14 sub-groups. The dissimilarity coefficient was calculated by Jackard IJ distance analysis, and result showed value ranged from 0 to 1. According to this dissimilarity coefficient we can understand the dendrogram and their relatedness. So, highest diverse genotypes can be used as parents in breeding programme. The average of dissimilarity coefficient varies from 0.7689 to 0.5079. The total average of dissimilarity coefficient of all sixty five

cultivar is 0.6119. The dissimilarity coefficient varied from the largest value 0.9429 between the cultivar LC-4 and IR 82635-B-B-47-1 followed by cultivar value 0.9286 between the cultivar OR 1946-2-1 and UPLRI-7 which shows high similarity between them and it may be expected that both of them may have arouse from the same parents. The lowest value 0.150 was found between REWA 1208-15 and PAU 3832-79-4-3-1. Similar result was repoted by Lapitan *et al.* (2007) and Siva *et al.* (2010).

CONCLUSION

Twenty random SSR markers were used out of which nineteen were found polymorphic. A total of fifty two alleles were detected by 19 polymorphic markers across sixty five rice genotypes with an average of 2.7 alleles per polymorphic marker. The amplicon size varied from 50bp produced by RM84 to 290bp produced by marker RM424. PIC value ranged from 0.032 to 0.588 and marker RM231 was found to be the most appropriate marker to discriminate among the rice genotypes owing to the highest PIC value of 0.588. The genetic divergence study grouped sixty five rice genotypes into nine clusters in which cluster IB-1a had maximum thirty-one genotypes followed by cluster IB-1b and cluster V. On the basis of dendrogram the highest similarity observed between cultivar Rewa 1208-15 and PAU 3832-79-4-3-1 followed by LC-15 and LC-16, BAU 411-05 and IR 82635-B-B-145-1 and BVD 111 and LC-11. The most diverse cultivar was IR 82635-B-B-47-1 and OR 1946-2-1. The highest dissimilarity coefficient value was observed between the cultivar LC-4 and IR 82635-B-B-47-1 (0.0429) and between OR 1946-2-1 and UPLRI-7 (0.9286) whereas lowest value was seen between REWA 1208-15 and PAU 3832-79-4-3-1 (0.150) showing highly diverse genotypes. Thus, these accessions were genetically diverse and could be directly utilized in hybridization programme for improvement of yield related traits or to execute efficient selection in highly segregating generations.

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