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GENETICS

Genetic diversity studies in rice (Oryza Sativa L.) using microsatellite markers

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ABSTRACT

The objective of present study was to evaluate the genetic polymorphism and identification of diverse parents among the 76 rice accessions using simple sequences repeat (SSR) markers. The accessions showed significant phenotypic variation for all the characters analyzed. The SSR Markers were highly polymorphic across all accessions and altogether 79 alleles were detected. The overall Polymorphic information content (PIC) value ranged from 0.26 to 0.65 with an average of 2.82 per locus indicting high level of genetic variation. The cluster analysis showed the rice germplasm accessions grouped in to two major groups and 14 subgroups. The pair-wise genetic dissimilarity co-efficient indicated that the highest dissimilarity was obtained between cultivar B.3688-TB and IR.67017-1(0.1935) followed by cultivar Badi Kodi and Changhat (0.3333). These grmplasms were showing wide genetic divergence among the constituent in it and may be directly utilized in hybridization programme for improvement of yield related traits. The markers RM 413, RM 481, RM 206 and RM 20 produced a maximum of four alleles. These microsatellite markers could serve as a powerful tool in selecting genetically diverse germplasm accessions, to execute efficient selection in highly segregating generations.

Highlights

- The PIC value indicates that all these markers were highly informative and capable of distinguishing between genotypes.
- The SSR markers are neutral and co-dominant and could be a powerful tool to assess the genetic variability of cultivars.

Keywords: Molecular diversity, Oryza sativa, PIC, alleles, SSR

Rice is the most important staple food grain and stands next to wheat in the global food grain production. India has the largest area under rice, about 44.6 M.ha of land with a production of about 106.19 Mt. (MoA 2014). The rice germplasm is a rich reservoir of useful genes that rice researcher can harness for rice improvement programme. Genetic variability exists among rice germplasm leaving a wide scope for crop improvements. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. It contributes to monitoring germplasm and can also be used to predict potential genetic gains. Studies have shown that indigenous crop varieties, traditionally cultivated and maintained by farmers contain high level of genetic diversity and can serve as potential genetic resources for improving yield, resistance to pests and pathogens, and agronomic performance

(Mandel et al., 2011, Vung et al., 2012, Bidhan ,2013). Similarly, quantification of genetic diversity existing within and between groups of germplasm is important and the crosses between parents with maximum genetic divergence are generally the most responsive for genetic improvement (Arunachalam 1981) for realizing higher heterosis and obtaining superior recombinants. Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Several molecular markers viz. RFLP, RAPD, SSRs, ISSRs, AFLP and SNPs are presently available to assess the variability and diversity at molecular level (Kulsum et al., 2011). DNA based SSR markers are considered most effective tool for identifying variation among germplasm due to their multiallelic nature, high reproduciability, co-dominant inheritance, abundance, stability and extensive genome coverage thus being widely applied in genetic diversity analysis especially in identification of species with closer genetic relationship (Devi et al., 2012). In this investigation, we selected 76 rice germplasm accessions with the objective of identifying genetically diverse rice germplasm, particularly to exploit maximum heterosis and recombination advantage in future breeding programmes. Special selection emphasis was given to yield and yield components.

Materials and Methods

Plant materials and field evaluation of morphological traits

The experiment was carried out during kharif season 2013 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The experimental seed material comprise of 76 rice germplasms collected from IRRI, Philippines; NBPGR, New Delhi; CRRI, Cuttack; NDUAT, Faizabad; BAU, Ranchi and NRCPB, New Delhi The nursery was sown on June10, 2013 and 21 days old seedling was transplanted in a randomized block design (RBD) with three replications. Each plot consisted of five rows of 1.5m length with spacing 15 × 20 cm. The recommended packages of practices were used for raising a good crop growth. Observations were recorded on days to 50% flowering, days to maturity , plant height (cm) , panicle length (cm), number of effective tillers/plant, panicle weight , total number of grains/panicle , filled grains/ panicle, spikelet fertility percentage, test weight (g), and grain yield/ plant (g). The statistical analysis means, range and coefficient of variations were carried out for all the traits.

Genomic DNA extraction and PCR amplification

Genomic DNA was extracted from the leaf of 10-15 days young plant by using CTAB DNA extraction method (Doyle and Doyle, 1990. DNA samples were diluted to 10 ng/µl. The SSR were chosen on the basis of their published molecular weight, reliability of amplification signal and polymorphism information content. The DNA was quantified spectrophotometrically (PerkinElmer, Singapore) by measuring A260/A280 and DNA quality was checked by electrophoresis in 0.8% agarose gel. A total of 95 SSR markers, selected from www.gramene.co.in and were synthesized by Eurofins Genomics (Bangalore, India) , and were initially evaluated by using five most morphologically diverse lines for their capability to amplify clear, reproducible, polymorphic DNA bands. Later, 28 polymorphic markers were selected for the analysis of all the 76 genotypes (Table 1). The 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 (Mastercycler gradient, Eppendorf). Thermal cycling program involved an initial denaturation at 940C for 45 sec, annealing at 20c bellow Tm of respective primers for 30 sec, primer extension at 72 0C for 30 sec, followed by a final extension at 72 0C 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

Tabel 1. List ofSSR markers, sequence, linkage group, number of alleles and their PIC used in genetic diversity.

Sl.No.	Primer name	Forward sequence	Reverse sequence	Chr. No.	No. of alleles	PIC value
1	RM259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	1	2	0.34
2	RM297	TCTTTGGAGGCGAGCTGAG	CGAAGGGTACATCTGCTTAG	1	3	0.52
3	RM580	GATGAACTCGAATTTGCATCC	CACTCCCATGTTTGGCTCC	1	4	0.34
4	RM279	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	2	2	0.37
5	RM7636	TCACTAACACAGGCAGAGCG	ACCAACTCAAGGCGAAGATG	2	3	0.41
6	RM53	ACGTCTCGACGCATCAATGG	CACAAGAACTTCCTCGGTAC	2	2	0.37
7	RM514	CCGAGGAGAGGAGTTCGAC	GTGCCAATTTCCTCGAAAAA	3	2	0.37
8	RM251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC	3	3	0.45
9	RM280	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG	4	3	0.51
10	RM131	TCCTCCCTCCCTTCGCCCACTG	CGATGTTCGCCATGGCTGCTCC	4	2	0.56
11	RM307	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	4	3	0.40
12	RM13	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG	5	3	0.52
13	RM413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	5	4	0.56
14	RM31	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG	5	2	0.36
15	RM3	ACACTGTAGCGGCCACTG	CCTCCACTGCTCCACATCTT	6	2	0.36
16	RM276	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	6	3	0.55
17	RM11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	7	2	0.37
18	RM481	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG	7	4	0.65
19	RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	8	3	0.58
20	RM264	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC	8	3	0.50
21	RM152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	8	3	0.56
22	RM242	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	9	3	0.57
23	RM316	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC	9	2	0.28
24	RM271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	10	3	0.57
25	RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	11	3	0.57
26	RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	11	4	0.63
27	RM20	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	12	4	0.64
28	RM17	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA	12	3	0.55

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

The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 50 base pairs DNA ladder. The SSR bands were scored manually for the presence (1) or absence (0) across all the genotypes. Only reproducible and clearly distinguished bands were taken into consideration. Polymorphic information content (PIC) value of a marker was calculated according to a simplified version of (Anderson *et al.*, 1993).

$$PIC = 1-\sum^{n}_{j=1} P^2ij$$

Where P_{ij} is the frequency of the j^{th} allele for the i^{th} marker, and summed over n alleles. Also,

average number of alleles, total heterosygozity and average PIC value were calculated..The pair-wise combinations of genotypes were employed to calculate Jaccard's similarity coefficient (GS)= a/(n-d), where a is the number of positive matches, n is the total sample size, and d is the number of negative matches (Jaccard 1908). This matrix was subjected to cluster analysis by the unweighted pair-group method (UPGMA; Sneath and Sokal 1973) and the dendrogram was constructed using the SAHN module of NTSYSpc software package. The dissimilarity matrix, genetic distances and cluster analysis were performed by Numerical Taxonomy and Multivariate Analysis system, Version 2.1 (NTSYSpc; Rolhf 1998).

Results and Discussion

SSR diversity and genetic relationship among rice genotypes

The ninety-seven markers were used across the seventy-six rice genotypes for their characterization and discrimination. Among these twenty-eight were polymorphic and used in present study. The remaining sixty-nine were showing monomorphic banding pattern and they were excluded. Altogether 79 alleles were detected at the loci of twenty-eight microsatellite markers across 76 rice genotypes. The number of alleles per marker locus varied from 2 to 4 with an average of 2.82 alleles per marker (Figure 2). The PIC values of the polymorphic markers

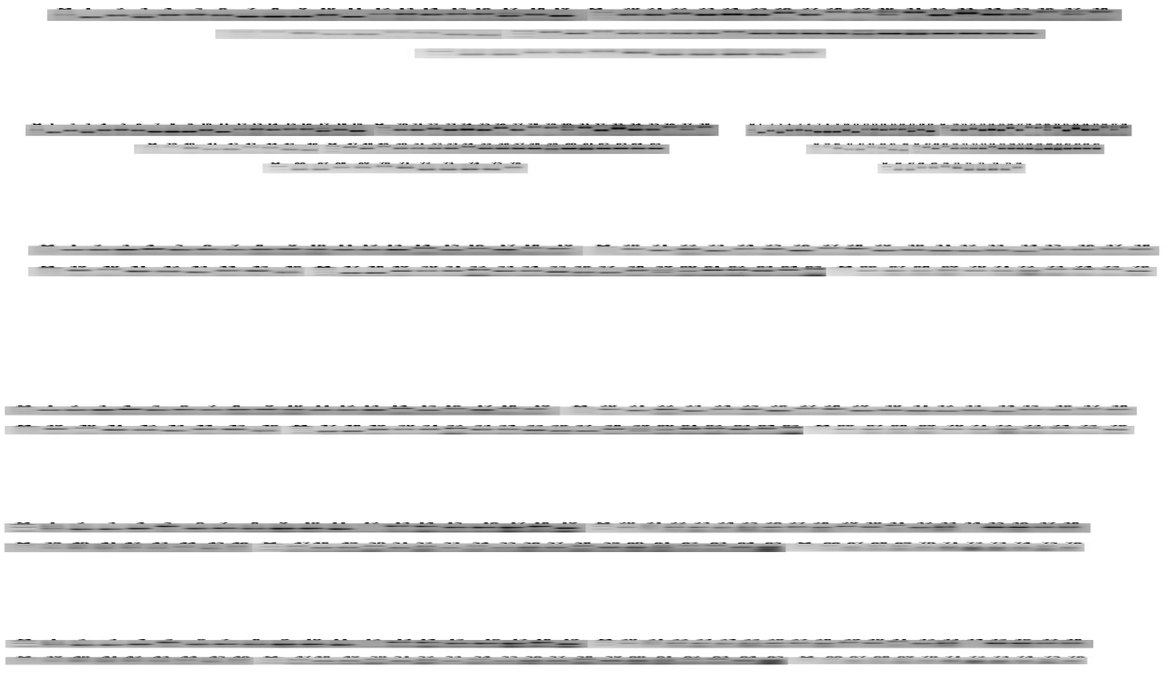

Figure 1. Separation of alleles on agarose gel followed by staining with ethidium bromide and visualized under UV light. PCR product were amplified with SSR markers RM297(A), RM206 (B) , RM 413 (C). M is 50 bp DNA size marker and numbers 1-76 represent 76 rice germplasm accessions as described in Table 2.

Table 2. Variation formorpho-physiological traits in seventy six rice germplasm accessions.

Sl. No	Genotypes	Days to 50% Flowering	Days to Maturity	Plant height (cm)	Panicle Length (cm)	Ears per Tiller	Panicle Weight (g)	Filled Grains per panicle	Total Grains per plant (g)	Spikelet fertility %	Test Weight (g)	Yield per Plant (g)
1	Dular	74.0	103.0	114.61	11.95	7.07	2.44	60.02	69.28	86.63	24.69	11.14
2	FR13A	149.0	193.0	96.90	19.46	8.28	3.03	95.98	108.25	88.66	21.15	17.48
3	FR13B	161.0	196.67	107.34	16.65	7.49	3.17	89.64	100.60	89.11	14.91	17.32
4	Karkati 87	128.0	166.33	164.66	27.21	14.73	3.69	142.33	156.03	91.22	27.82	29.39
5	Birpala	159.33	197.33	102.42	11.99	7.91	2.29	71.31	82.79	86.20	20.61	11.58
6	Tetep	91.0	127.67	137.09	6.74	5.45	1.75	52.95	66.74	79.38	18.44	7.45
7	HeenSulai	139.0	147.0	161.37	23.32	12.12	3.44	144.34	155.02	93.16	22.44	22.88
8	Madal	154.33	173.33	138.51	11.77	5.37	2.40	79.35	87.76	90.42	24.41	11.97
9	MahaDikwee	153.0	172.67	193.66	18.13	10.07	2.81	88.64	95.95	92.40	14.73	17.93
10	Ausboro	94.0	123.0	142.38	10.81	5.03	1.73	46.46	58.31	79.73	24.57	6.92
11	Jaldungi	152.3	194.0	140.43	22.46	11.59	3.19	102.57	113.15	90.68	18.02	20.48
12	Nirboi	153.33	191.33	142.26	18.14	10.28	2.66	96.65	104.99	92.06	12.79	17.21
13	CR 1009	149.0	187.33	97.11	10.10	6.32	2.14	66.33	75.89	87.45	12.95	9.19
14	HouraKani	154.67	192.0	122.83	9.54	4.99	2.13	54.51	69.42	78.51	16.97	7.83
15	Naldak	154.0	182.0	144.07	12.44	5.74	2.57	77.20	87.38	88.39	32.59	11.39
16	Lunishree	146.0	185.67	137.42	21.85	9.16	3.57	98.83	108.72	90.91	19.34	20.43
17	Swarna Sub-1	131.0	182.0	84.24	18.79	11.73	2.01	90.02	98.16	91.71	11.69	19.43
18	Sambha Sub-1	123.33	162.0	85.78	23.07	12.17	2.74	120.28	143.80	83.61	11.69	24.02
19	Kariyawa	135.33	177.33	168.67	30.54	15.89	4.15	164.48	194.96	84.41	24.81	32.0
20	Godaheenate	127.33	172.67	174.63	19.54	11.99	3.18	108.85	125.30	86.91	21.73	19.63
21	S-155	71.33	107.0	78.22	13.12	7.56	2.85	80.67	109.33	74.28	17.66	12.77
22	TSAO WANCHING	59.0	104.67	107.94	15.99	8.80	2.89	95.33	108.33	88.33	22.42	15.44
23	JC-1	72.0	104.0	137.17	30.89	14.88	4.31	152.52	157.67	96.91	22.63	32.45
24	Lumbini	114.33	104.33	169.44	31.74	14.74	4.63	205.08	224.00	91.61	17.04	34.63
25	Madhukar	75.00	105.67	93.15	12.78	5.32	2.06	76.07	116.83	65.19	20.42	10.87
26	N-22	59.67	104.67	118.29	24.84	14.88	3.12	138.78	143.74	96.57	23.89	26.18
27	Binulavan	62.0	108.0	123.78	24.68	13.55	3.27	105.68	134.44	78.79	17.29	24.18
28	Kala-rata 1-24	75.33	102.67	123.44	13.15	5.57	2.07	77.55	93.67	83.00	23.46	11.31
29	Tatan	121.33	169.00	130.25	12.96	5.49	1.89	71.24	92.67	77.54	19.37	11.017
30	Bapkaribuna	122.33	168.33	137.25	12.79	5.57	1.68	62.57	84.33	74.52	16.87	10.20
31	Cota	113.0	157.67	166.03	25.04	15.09	3.18	141.63	164.33	87.20	22.63	27.48
32	Khaijan	117.0	157.33	136.12	13.21	4.28	1.23	50.26	68.17	73.65	23.48	8.19
33	Kamnam	68.0	105.67	132.78	13.92	4.70	2.00	52.0	85.00	61.29	22.38	8.47
34	Lemoht	60.33	103.67	73.55	23.24	11.91	3.25	123.55	143.17	86.41	22.77	21.31
35	Latsika	60.0	100.33	89.57	17.25	6.84	2.74	91.62	105.00	87.29	20.84	14.42

36	A k i t a Komachi	57.33	100.0	59.33	23.48	13.11	3.15	127.58	136.00	93.82	17.22	23.93
37	M-202	56.00	98.0	60.61	25.07	5.73	2.42	84.99	95.67	88.86	19.56	11.29
38	MR-Amroo	57.33	106.33	70.11	13.06	5.77	2.18	63.73	78.00	82.35	23.89	10.30
39	Nipponbare	55.00	104.0	57.92	14.67	5.52	2.64	74.14	90.0	82.41	20.91	12.45
40	AsamiDhan	73.0	106.0	109.36	19.07	7.25	2.55	89.78	152.74	58.79	27.53	16.71
41	Changhat	116.0	156.67	144.17	18.74	8.74	3.03	78.22	92.18	84.94	22.69	16.38
42	BadiKodi	120.33	153.33	148.69	18.48	9.71	2.59	85.32	108.67	78.71	23.19	18.18
43	Dub Celong	58.67	156.67	111.16	25.70	15.67	3.77	131.58	155.0	85.09	24.39	25.85
44	Savitri	58.0	150.33	99.35	27.18	17.27	3.94	141.99	155.87	91.30	17.81	27.58
45	AshuBhajna	56.0	122.0	140.88	25.63	13.27	2.97	141.24	161.50	87.52	16.51	26.40
46	Tundahiya	61.0	130.33	128.53	20.33	10.3	2.61	91.17	100.33	91.10	15.96	18.04
47	CB05-753-3	93.0	110.33	95.33	23.97	8.0	3.77	177.0	197.67	89.30	25.00	13.76
48	IR.73000-98-1-2-1	86.0	105.67	95.50	26.17	6.0	3.75	137.0	150.0	91.09	21.73	10.29
49	Brown gora	80.33	101.0	97.27	25.60	6.0	3.64	77.67	88.67	87.23	23.83	18.04
50	I R . 8 2 9 1 2 - B-B-10	89.67	110.33	107.50	25.77	7.67	3.72	131.67	136.67	96.35	24.34	16.71
51	I R . 8 2 3 1 0 - B-B-67-2	76.0	96.00	103.67	28.67	7.0	3.92	140.33	160.67	87.36	24.76	23.36
52	UPRI 2012-16 IV7	89.0	109.67	130.67	27.67	8.67	4.16	170.33	187.33	90.82	20.12	20.11
53	CR.3488-1-2-1-2 IV7	88.33	109.67	115.67	25.67	6.67	3.62	142.00	157.0	90.39	22.23	15.27
54	I R . 8 2 9 1 2 - B-B-14	75.33	97.33	108.47	26.73	8.67	2.75	101.33	115.67	87.60	26.84	16.98
55	I R . 8 3 9 2 6 - B-B-71-4	80.67	101.0	123.43	25.43	8.67	2.74	143.67	150.33	95.33	24.54	18.64
56	CT.15678-2-3-3-1	87.33	108.33	102.63	25.73	10.67	3.49	172.0	191.0	90.12	21.99	21.29
57	B . 1 0 5 8 0 E - KN-28	83.33	106.67	113.40	27.17	7.33	4.86	171.67	185.67	92.44	31.08	24.11
58	I R . 8 2 6 3 5 - B-B-93-2	80.67	105.0	118.67	24.77	6.00	3.03	156.3	169.00	92.34	23.30	13.64
59	BVD.109	79.00	99.0	114.33	23.17	10.33	1.49	79.0	85.67	92.19	22.20	10.26
60	IR.71146-97-1-2-1-3	81.33	102.33	109.60	23.83	6.67	3.51	150.3	161.67	92.96	20.33	7.39
61	I R . 8 2 6 3 5 - B-B-143-1	75.33	96.67	133.67	25.60	11.33	3.05	137.3	152.67	89.79	20.06	12.69
62	BAG .408-05	79.33	102.33	104.33	26.63	12.00	2.81	123.3	148.0	83.40	24.29	16.44
63	I R . 8 2 9 1 2 - B-B-2	82.33	103.67	115.90	27.47	9.67	2.81	87.0	97.33	89.28	26.06	13.51
64	I R . 8 2 9 1 2 - B-B-7	75.0	97.33	116.03	26.67	11.0	3.44	116.33	134.0	86.74	26.17	11.18
65	IR.839299-B-B-132-3	76.67	95.33	99.33	20.47	4.67	3.41	84.0	124.00	67.24	31.14	5.26

66	IRAT-112	74.33	100.33	129.27	29.83	9.67	3.21	137.0	162.33	83.17	19.98	17.83
67	IR.87756-20-2-2-3	75.00	96.0	104.50	26.73	9.33	3.83	135.3	156.33	86.59	15.04	12.95
68	IR.67017-124-2-4	82.67	106.0	111.90	27.57	6.00	4.38	154.3	196.67	78.45	24.34	12.95
69	B.3688-TB-25-MR-2	82.33	107.67	118.40	28.10	5.33	3.92	132.67	147.67	90.077	27.81	10.89
70	BP.19768-2-3-7-78-1-1-	81.00	102.67	127.30	24.47	6.67	4.08	111.3	127.00	87.61	30.16	15.86
71	IR.78933-B-24-B-B-4-	86.67	106.33	135.90	28.13	7.67	4.84	143.67	176.67	81.31	24.91	23.66
72	BP.1351D-1-2-PK-3-1	76.00	97.0	124.50	27.23	6.00	3.25	134.33	168.33	78.32	23.24	15.37
73	RR.617-B-3-3	110.67	132.0	89.33	25.01	7.67	2.78	218.0	243.00	89.71	16.01	12.06
74	CR.3423-1	84.67	108.0	108.47	24.67	8.67	3.99	180.33	195.00	92.46	16.14	15.44
75	OR.1946-2-1	82.00	102.67	100.67	27.20	6.67	3.32	105.33	124.00	84.67	23.31	8.99
76	Kala bunde	102.33	124.67	121.33	27.20	7.0	4.55	161.0	189.33	85.27	24.83	23.05
	Mean	95.32	128.34	118.16	21.64	8.85	3.08	113.11	130.76	85.90	21.64	16.71
	C.V.	1.57	1.77	3.94	8.02	16.68	13.83	13.73	12.55	4.99	3.21	15.16
	S.E.	0.8644	1.31	2.69	1.00	0.85	0.25	8.97	9.48	2.48	0.40	1.46
	Range Lowest	55.0	95.33	57.92	6.74	4.28	1.23	46.46	58.31	58.79	11.69	5.26
	Range Highest	161.0	197.33	193.66	31.74	17.27	4.86	218.0	243.00	96.91	32.59	34.63

ranged from 0.267 (RM 316) to o.654 (RM 481) with an average PIC value of 0.47. The markers RM 413, RM 481, RM 206 and RM 20 produced a maximum of four alleles. These findings are Conformity with the earlier reports of (Sajib *et al.*, 2012, Singh *et al.*, 2013 and Thomson *et al.*, 2006). Clustering of germplasm accessions based on 28 polymorphic markers produced two major clusters at the dissimilarity coefficient of 0.26 that is Cluster I and II. Cluster I further differentiate in to two sub- clusters viz. IA and IB. Sub cluster IB further differentiated in to two mini clusters viz. IB-i (4) and IB-ii was monogenic(1). Sub-cluster IA also differentiated in to two mini clusters viz. IA-i and IA-ii (2). Cluster IA-i had two clusters IA-ia (5) and IA-ib (8). Cluster II also further differentiated in to two sub-clusters viz. IIA and IIB. Cluster IIB includes IIB-I (3) and IIB-II was monogenic (1). Cluster IIA consists of two mini clusters IIA- i (48) and IIA-ii (4) germplasm accessions. Jackarsd's similarity matrix was used to generate a dendogram to obtain the clustering of genotypes (Figure 2) The Jackard dissimilarity coefficient value shows close to 1 indicates maximum similarity and the values close to '0' show maximum dissimilarity. Highest similarity was found between cultivar CB05-753-3 and Tundahiya (0.94) , followed by cultivar Brown gora and IR.73000-9 (0.840. The highest dissimilarity was found between cultivar B.3688-TB and IR.67017-1(0.1935) followed by cultivar Badi Kodi and Changhat (0.3333), and between cultivar IR.82912-B and BAG .408-0 (0.3784) indicated that these were genetically diverse germplasm accessions and may be directly used in hybridization programme for yield improvement. The total average of dissimilarity coefficient of all 76 cultivar is 0.70.

Morphological variations in rice germplasm accessions

Eleven morphological and yield related traits were recorded for assessment of phenotypic variability among the 76 rice germplasm. The germplasm used in the present study showed significant phenotypic

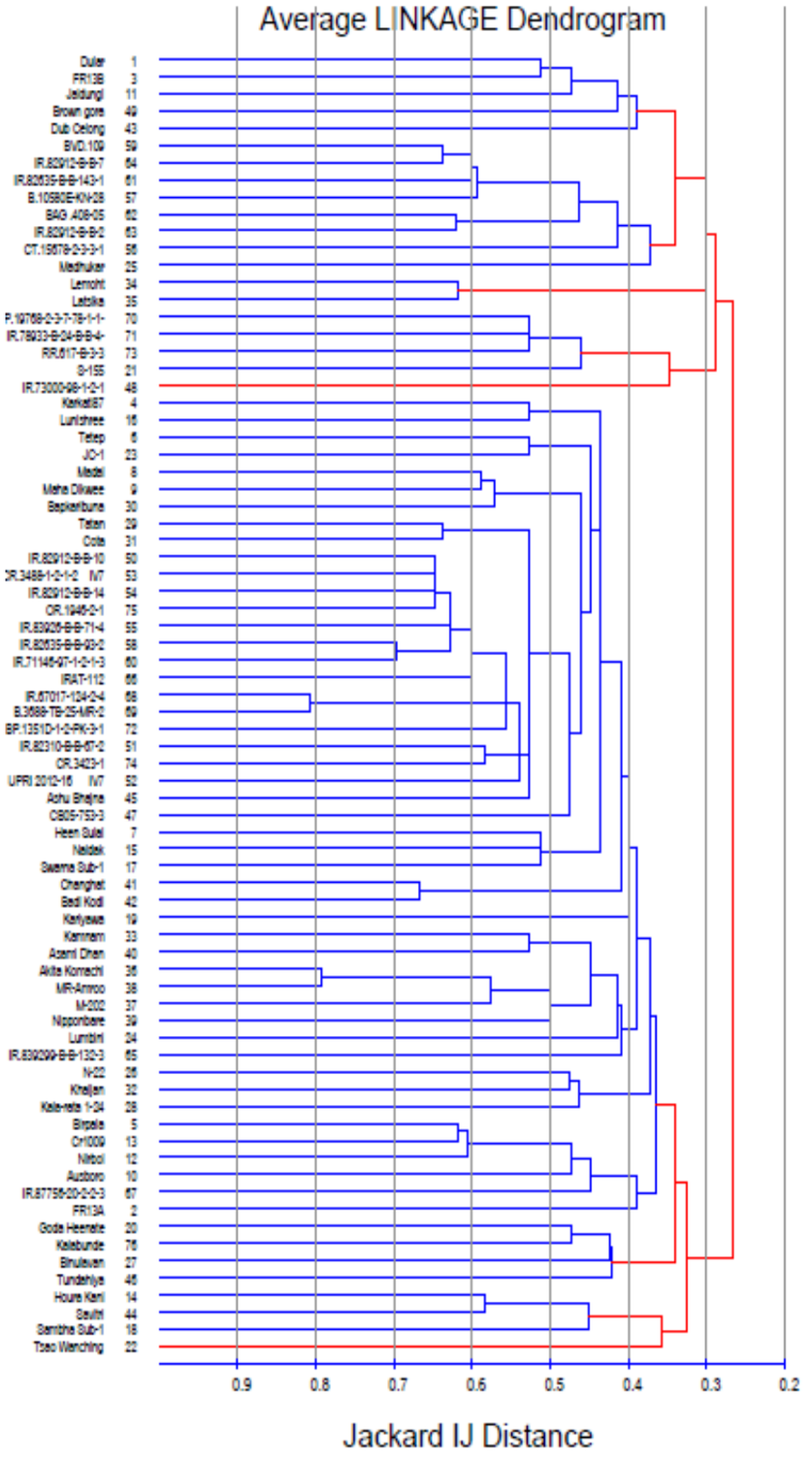

Figure 2. UPGMA dendrograme based on Jaccard's similarity coefficient showed the clustering of 76 rice germplsm accessions.

variation for all the characters analysed. The basic statistical analysis of the results with respect to yield and yield associated traits are presented in Table 2. There was wide range of variation in a present set of material. Maximum number of effective tillers per plant was recorded for Savitri (17.27) while minimum number of effective tillers per plant was for Khaijan (4.29). Days to maturity was found maximum in Birpala (197 days), while minimum in IR. 839299-B-B-132-3 (95 days). A large variation was observed in plant height which ranged from 57 cm (Nipponbare) to 193.66 cm (Maha Dikwee). Panicle length has also shown a large variation ranging from 6.73 (Tetep) to 31.74 (Lumbini). A large variation in filled grain per panicle and total grain per panicle was ranged from 46.45 (Ausboro) to 218 (RR.617-B-3-3) and 58.31 (Ausboro) to 243 (RR. 617-B-3-3) respectively. Spikelet fertility was recorded maximum in JC-1 (96.91) and minimum in Asmidhan (58.79). The grain yield per plant was found highest in Lumbini (34.63g) followed by JC-1(32.4g) and karkati (29.39g). Significant variation was also observed for days to 50% flowering, ear per tiller, panicle weight and test weight. Chouchan et al (2014) and Singh et al (2013) also observed highest morphological variation for leaf width, plant height, panicle length, filled grain per panicle, number of total grain per panicle, spike fertility, test weight, yield per plant and lowest for leaf length and number of effective tiller per plant.

Conclusion

The present study revealed a wide variation among the germplasm accessions. The result indicated that the SSR marker is neutral and co-dominant and could be a powerful tool to assess the genetic variability of cultivars. The PIC value indicates that all these markers were highly informative and capable of distinguishing between genotypes. The total average of dissimilarity co-efficient of all 76 cultivar is 0.70. The highest dissimilarity was found between cultivar B.3688-TB and IR.67017-1(0.1935) followed by cultivar Badi Kodi and Changhat (0.3333), and between cultivar IR.82912-B and BAG .408-0 (0.3784) 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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