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Molecular Characterization of Rice (*Oryza sativa* L.) Landraces from Southern Karnataka by Using Simple Sequence Repeat (SSR) DNA Markers

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ABSTRACT

Genetic diversity among rice landraces using simple sequence repeat (SSR) DNA markers was taken up to find the extent of variation. Forty six rice landraces (*Oryza sativa* L.) were collected from South Karnataka locations for genetic diversity analysis using seventy four SSR markers distributed on twelve rice chromosomes. Upon PCR amplification the alleles were separated by using MultiNa, a microchip electrophoresis system. A total of 1301 amplicons were generated among the landraces from marker analysis. The number of alleles per locus ranged from 4 to 34 with an average of 18 polymorphic alleles per locus. PIC value varied from 0.59-0.96 with an average of 0.87 per primer. It was found to be highest for the primers RM 2190, RM 6673, RM 7642 and RM 1159 while the lowest value was for RM263 marker. The major allele frequency ranged from 0.06 to 0.54 with mean of 0.21 and the average gene diversity was 0.88 with the range between 0.66-0.97. The dendrogram constructed based on SSR markers differentiated 46 landraces, which were distinctly clustered into three main clusters *viz.*, Cluster A, B and C. Three main clusters comprised of 26, 17 and 5 landraces, respectively, that showed a high degree of diversity among the cultivars. The results of the genetic diversity information will be very useful for proper identification, characterization, and conservation of rice landraces and also serves as the sources for parental selection in hybridization.

Keywords: Land races, genetic diversity, rice, SSR markers, polymorphism information content (PIC)

DIVERSITY is the main basis for selection for crop improvement. Rice has unlimited germplasm collected across the world and preserved from wide range of eco-systems. Rice is one widely cultivated major cereal crop in Asia. It is one of the most diversified crop species due to its adaptation to a wide range of geographical, ecological and climatic regions in the world. India is remarkably rich in rice diversity, including cultivars, landraces, wild and weedy relatives. Growth and development of agricultural resources is mostly depends on genetic diversity among different crop plants and it is estimated that not even 15 per cent of the potential diversity has been utilized. This implies that thousands of valuable allelic variations for traits of economic significance remain unutilized in commercial crop improvement and production (Hossain *et al*., 2007). Northeast India is a well-known centre of diversity for rice (*Oryza sativa* L.) characterized by varied topography and climatic conditions, which contributes to the valuable genetic resource for future crop improvement (Anupam *et al.*, 2017). Landraces are precious genetic resources for any crop improvement program, because they contain huge genetic variability which can be used to complement and broaden the gene pool of advanced genotypes (Kobayashi *et al*., 2006). Therefore, landraces of distinct genetic structure are a good promise for the future rice crop improvement. Landraces of rice played a very important role in the local food security and sustainable development of agriculture, in addition to their significance as genetic resource for rice genetic improvement (Tang *et al*., 2002). They have provided “adaptability genes” for specific environmental conditions. Incorporation of such adaptability genes from landraces only could ensure optimum grain yield for the specific regions. Thus to maintain crop diversity for crop improvement program collection, characterization and conservation of traditional landraces is vital. Genetic diversity is a ubiquitous feature of all species in nature and plays an important role in the selection of parents having wider variability for different characters.

Genetic diversity is generally assessed by morphological traits and molecular markers. Molecular markers based on PCR are powerful tools in the assessment of genetic variation. In the elucidation of genetic relationships within and among species and have demonstrated the potential to detect genetic diversity and aid in the management of plant genetic resources (Virk *et al*., 2000; Song *et al*., 2003, and Teixeira da Silva, 2005). Among various PCR based markers, simple sequence repeat (SSR) markers are more popular in rice because they are highly informative, mostly mono-locus, co-dominant, easily analyzed and cost effective (Hittalmani *et al.* (2013); Shivapriya & Hittalmani (2006) and Gracia *et al*., 2004). Thus, in the current study SSR markers were used to assess the genetic diversity among rice landraces collected from Southern Karnataka region.

MATERIAL AND METHODS

Plant materials: The experimental materials comprised of 46 rice landraces along with two released varieties as control *viz.*, MAS-26 and MAS-946 collected from different agro-ecological regions of Karnataka and are maintained in the Department of Genetics and Plant Breeding, UAS, Bangalore. The details of rice landraces used in the study is presented in Table I.

TABLE I

List of rice landraces used for present study

Sl.No.	Landraces	Sl.No.	Landraces
1	Bangara Sanna	25	Kirwana
2	Bilidoddi Buddha	26	Karigaj
3	Bilidoddi Marthylaya	27	Kari Kandadaga
4	Bangara Kaddi	28	Mysore Mallige
5	Chithaga	29	Mallige
6	Dodda Mulare	30	Mugad Sugandha
7	Gamnada Batta	31	Matakka Ra
8	Giddaralia Kamala	32	Jiddu Batta
9	Gidda Batta	33	Muttina Sanna
10	Halubulla	34	Neergula Batta
11	Halu Gidda	35	Naga Batta
12	Honne Kattu	36	Neergoli
13	Jeerige Salaiah	37	Parimala Sanna
14	Jenugudu	38	Rat Bath
15	Kotambari Nelu	39	Raja Bhog
16	Kari Doddi	40	Sannaki
17	Kysare	41	Selam Sanna
18	Kaduvelpe	42	Sampige Batta
19	Kalikatesi	43	Sugandhi
20	Kappu Batta	44	Sampigeda
21	Kadulite	45	Sirsi Local
22	Kempu dadigida	46	Uduru Mallige
23	Kari Batta	1	MAS-26 (Varieties)
24	Krishna Leela	2	MAS-946 (Varieties)

DNA extraction

Young leaves were collected from each rice landrace for genomic DNA isolation. Leaf samples were finely powdered with liquid nitrogen for isolation of genomic DNA using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1987). The DNA quality was checked by electrophoresis in 0.8 per cent agarose gel and used for PCR amplification.

SSR analysis

Seventy four SSR markers were used to analyse the diversity of rice landraces (Table II). The primers were synthesised by Eurofins Genomics, Bangalore, India. The amplification was carried out in 10µl mixture containing 20ng DNA 1.0µl taq buffer, 0.5µl dNTPs, 0.20µl Taq DNA polymerase (MBI Fermentas, USA) and 0.8µl primers in a thermal cycler (Eppendorf). Thermal cycling program involved an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 2°C below Tm of respective primers for 30 sec, primer extension at 72°C for 1 min, followed by a final extension at 72°C for 5 min. The amplified PCR product with a DNA-500 separation buffer, marker solution kit (Shimadzu, Invitrogen) where size fractioned by using MultiNA, a microchip electrophoresis system.

Data analysis

Clearly resolved unambiguous PCR amplified bands were scored by MultiNa viewer for their presence or absence for each primer. The scores were obtained in the form of allelic value, which indicated the amplicon size of bands in each variety respectively.

TABLE II

Particulars of simple sequence repeat (SSR) markers used for diversity study

SSR Marker	Forward Sequence (5'-3')	Reverse Sequence (3'-5')	Chromosome Number	Annealing Temp. (C^0)	Expected Product Size (bp)	Observed Product Size (bp)
RM1013	GCTGCAATGTCTTTCACTGC	GGCTTTGGGGGAAATAGAAG	9	55	147	150
RM1328	GAATGGGATTAGACGATTTG	CCATGAGTGACATCAAAAGG	2	55	420	390
RM1817	TAGTATTCTTTCCTTACAGA	ATTGAAAACTTAACAAATAG	9	55	129	120
RM1026	GCCTCTGGCAGAATAGCATC	TATCACTTTGCTGCCTAGGC	9	55	164	154
RM6308	TCGACCTGGCTCTCCTCTAG	TATCAACCTGCTCCTCCTGG	3	55	104	120
RM7102	TTGAGAGCGTTTTTAGGATG	TCGGTTTACTTGGTTACTCG	12	55	169	170
RM7000	CCCTTCTTTTCAACTGAATA	TTGTAACAATGAACTCGTTC	3	55	138	150
RM6018	ATTAATACCGCTACCACGCG	TCCTCCTCCACCTCAATCAC	8	55	120	110
RM6022	ACCTGGACTCCATTACTCGC	AGAAGCTCACCTCGATGTGG	12	55	175	190
RM6404	GGGATGATGGATCGGGAG	CTACCAGCCTTGTTTCCTCG	10	50	150	145
RM1161	GCACTGCTGCTCCGAGTAGTACC	ATCCTCACTGCCCACCTGACC	6	55	80	80
RM6775	GCAGATCAAGTATGCCTGCC	TCGCTAGATAGGGGATGTGG	6	55	192	200
RM6306	CACCGGTCTAAGTCGACTCC	CCACTCGTTGTCGTCGTATG	12	50	88	90
RM7424	AGAAGCCCATCTAGCAGCAG	TCAAGCTAGCCACACAGCTG	9	50	82	82
RM6673	CATCGCATCGTATCGTATCG	GCTTCAAACACGCCTTCTTC	10	55	150	150
RM1099	AGATAGAAGGAGGGAAAGGCATGG	CTCCTTGGCTCACAATGCTTGG	9	50	82	90
RM3249	GCCCTTTTCTTCTCCACTCC	AGACACTGTCACAGCTTCAGC	9	55	151	160
RM5753	AACATGCTCAACTTCTGGGC	GCTAGGTACGATCCAGCTGC	6	55	201	200
RM1159	GTTGATGGTGTCAGCGAGAG	ACATTTGCACCACACCACAG	12	55	169	170
RM2136	CTTCCTCACCATCCGAGAGTTCC	AGCCAGCAAACGAACGACTAACC	11	55	136	140
RM2190	ACTGCAATAAATCCCAGTAA	ACTCAATTTCAACAATGGTG	9	55	148	150
RM8231	TGCTTCTTGTCAATTCGCC	CGACTCGTGGAGGTACGG	4	55	211	220
RM3529	CGCGCCACCTCGATATATAC	GCTCAGGTTAACCAAGGTGG	5	55	148	150
RM3646	ACTAGAGCACCCTCGCTGAG	CTCAGCCACCCCATCAAC	3	55	137	140
RM7389	AGCGACGGATGCATGATC	TTGAGCCGGAGGTAGTCTTG	3	55	111	115
RM7642	ACGAAATATCAGGGCACCTG	GTTGACTTTGGTCATGAGGG	3	50	194	200
RM3563	GTACGGAAAGACGAGAGATGC	CCCTTTTAGGTCGCAACTTG	10	50	116	220
RM8213	AGCCCAGTGATACAAAGATG	GCGAGGAGATACCAAGAAAG	1	55	177	180
RM6818	GTCGCATTCGTCTCCACC	ACCATTTCCAGATGACTCGG	6	55	130	140
RM84	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC	1	55	113	120
RM14	CCGAGGAGAGGAGTTCGAC	GTGCCAATTTCCTCGAAAAA	1	55	191	190
RM3431	ATCCAAATCCAATGGTGC	GCGAAAGGGAACATTCTG	6	55	161	170
RM10	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGGTCC	7	55	159	170
RM22	GGTTTGGGAGCCCATAATCT	CTGGGCTTCTTTCACTCGTC	3	55	194	190
RM18	TTCCCTCTCATGAGCTCCAT	GAGTGCCTGGCGCTGTAC	7	55	157	160
RM23	CATTGGAGTGGAGGCTGG	GTCAGGCTTCTGCCATTCTC	1	55	145	140
RM5095	CTATATGACTATGCGAATGG	ACAAATGCAACTAAGGTAGAA	10	55	182	180
RM5183	AATGAGCTAATGTTTCTAAG	AGCTTGAACCTTATATATTG	5	55	151	150
RM3525	ACACTCTCAGCTCATCAAGACC	GGGCAAGTGGTCAAATCTTG	3	55	179	180
RM3486	TCTCTTTTCCCTCCTTTCCC	GGCCTGCAAGAGGAGAAAAC	5	55	98	100
RM166	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG	2	61	321	330
RM240	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC	2	55	132	140

SSR Marker	Forward Sequence (5'-3')	Reverse Sequence (3'-5')	Chromosome Number	Annealing Temp. (C^0)	Expected Product Size (bp)	Observed Product Size (bp)
RM223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG	8	55	165	170
RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	11	55	157	160
RM13	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG	5	55	141	145
RM208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	2	55	173	180
RM319	ATCAAGGTACCTAGACCACCAC	TCCTGGTGCAGCTATGTCTG	1	55	134	130
RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	2	55	199	200
RM178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC	5	67	117	140
RM141	CACCACCACCACCACGCCTCTC	TCTTGGAGAGGAGGAGGCGCGG	6	55	136	150
RM163	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	5	55	124	130
RM154	GGTTCATGCACACCTGTAGTCC	GCACTCGTGAGAGAGTGATGC	2	61	183	190
RM138	GGACAGAATGTGAAGACAGTCG	ACTAATCCACCAACGCATCC	2	55	233	250
RM519	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC	12	55	122	120
RM526	CCCAAGCAATACGTCCCTAG	ACCTGGTCATGACAAGGAGG	2	58	240	230
RM151	AGCAGTAGCTGCATCGAAGG	GTATGTGCTCTTGCATTCTTGC	1	55	197	190
RM1003	GATTCTTCCTCCCCTTCGTG	TTCCTGTCAGAACAGGGAGC	1	55	128	135
RM302	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC	1	55	156	160
RM3825	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG	1	55	147	150
RM5552	ATCAGCCCAGAGGGAGTAAC	AGATTCTGGGATCCACGTTG	1	55	112	120
RM246	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	1	55	116	120
RM3542	CTCCATGGAAAGCTAGCCAG	AATCACCCTTTCAGTGCCTC	2	55	87	90
RM6838	ATTAATACCGCTACCACGCG	TCCTCCTCCACCTCAATCAC	8	55	120	125
RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	8	55	166	160
RM1896	CGTTTCACAATGTAAGACTT	CTCCGTTTTACAATGTAAGA	9	55	108	110
RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	11	55	147	150
RM4862	CAACTTTCTGGCATAAACTA	TGGTGAAAGATATTTCAGAC	11	55	164	160
RM7102	TTGAGAGCGTTTTTAGGATG	TCGGTTTACTTGGTTACTCG	12	55	169	170
RM1337	GTGCAATGCTGAGGAGTATC	CTGAGAATCTGGAGTGCTTG	12	55	210	215
RM155	GCAACACATCAAACTGCTGAATCG	CGTTAGGTGCGAACGAAGTTCC	12	55	255	260
TRS33	AAGAAGAAGCGTACGCATGAAT	GTCCTGGAGGGGAGGAGA	11	55	0	0
JJ133 T3	CTCTTGGTGATCTTTGTTAC	GGATGATGTGATCTGCAGAG	0	55	0	250
NMSM	CGAGAAGGACATCTGGTACG	GAGATGCTTGGATTTAGAAGAC	0	55	0	180

Polymorphic information content (PIC) values were calculated for each of the SSR loci using the formula developed by Nei *et al.* (2002).

$$PIC=1-\Sigma\ x^2_k\ /n$$

Where, x^2_k represents the frequency of the k^{th} allele, n represents the number of genotypes. The summary statistics on major alleles frequency, allele number, gene diversity and PIC values (Bostein *et al.,* 1980) were calculated using Power Marker version 3.2 (Liu and Muse, 2005) and Darwin version 5.0 (Perrier and Collet, 2006).

RESULTS AND DISCUSSION

Marker polymorphism and genetic diversity

Genetic diversity assessment of the landraces is essential component in germplasm characterization, conservation and to identify potential parents for crossing. There have been a number of studies that have reported on the assessment of genetic diversity in a relatively large set of cultivated germplasm in rice and other crops. This include diversity analysis of high yielding rice cultivars (Nei *et al*., 2002), aromatic rice (Nagaraju *et al*., 2002), indigenous

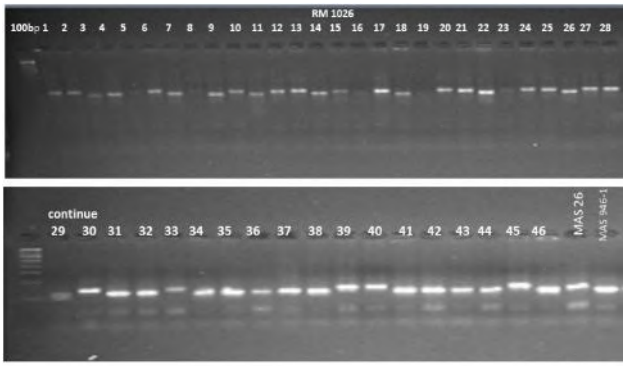

Fig.1: Rice landraces showing polymorphism by RM1026 marker

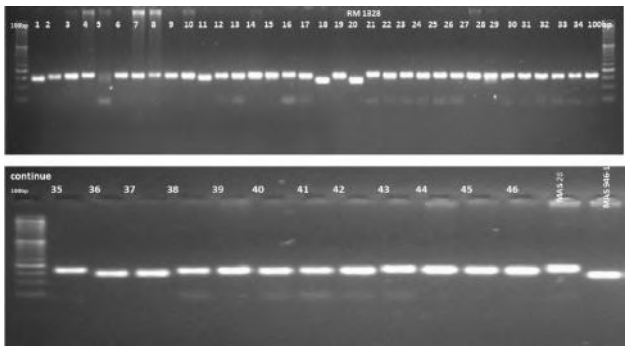

Fig. 2: Polymorphism of rice landraces as observed by the SSR marker RM1328

aromatic rice (Joshi and Behera, 2006) and even lowland rice (Bhuyan *et al*., 2007) using various molecular finger printing techniques like RFLP, RAPD, SSR, and AFLP. In the present study, a total of 74 SSR markers were used for PCR amplification on 46 landraces and two controls. Out of 74 SSR Markers, 73 markers clearly amplified and all markers generated polymorphism among the rice landraces. A total of 1301 amplicons were produced among the landraces (Fig. 1 and 2). Microsatellites are among the most widely used DNA marker for many purposes such as diversity, genome mapping, and varietal identification. (Teixeira da Silva, 2005). As SSR markers are neutral and co-dominant, were used as powerful tool to assess the genetic variability of the cultivars and landraces. The present study revealed exceptionally high genetic variation, with an average of 18.0 polymorphic alleles per marker and the number of alleles per locus ranged from 4 to 35 (Table III). These SSR markers exhibited 100 per cent polymorphism. Similar genetic diversity studies in rice revealed 6 to 15 alleles per locus with an average of 8.44 in twenty four rice germplasm accessions using nine SSR primers (Furkanur *et al* 2015, Babu *et al*., 2014, Choudhury *et al*., 2013 and Etemad *et al.,* 2012). Presence of high polymorphic alleles indicates the possibility diverse genetic background of the rice landraces used in the present study and the power of SSR markers differentiation at allelic level.

PIC value, a parameter associated with the discriminating power of markers, varied from 0.59-0.96 with an average of 0.88 per primer. Generally, PIC values higher than 0.5 indicates high level of polymorphism. The presence of more than 0.5 PIC value indicated in our results shows that SSR markers used in this study were highly informative in differentiating the landraces. It was found to be highest for the SSR primers RM 2190, RM 3249, RM 8213 and RM 3431 while the lowest value was for RM 263. The high PIC value observed among the rice landraces indicated their wide genetic diversity and lower PIC value may be the result of closely related landraces. According to the earlier reports, PIC values ranged from a low of 0.24 to a high of 0.92 and averaged 0.61 (Jain *et al*., 2004), and 0.19 to 0.90 with an average of 0.75 (Borba *et al*., 2009). The average PIC value of 0.44 was observed among 43 Thai and 57 IRRI germplasm of rice (Chakhonkaen *et al*., 2012). In another study, an average PIC value of 0.45 was observed among the 183 Indonesian rice landraces on the Islands of Borneo (Michael *et al*., 2009). A slightly lower genetic diversity was reported in 375 Indian rice varieties collected from different regions of India with 36 polymorphic SSRs in which they detected 2.22 alleles per locus and an average PIC value of 0.25 (Singh *et al*., 2013). Similarly, Anupam *et al.,* 2017 reported 2 to 5 alleles per locus with an average of 2.9 and PIC value per locus of 0.059 (RM537) to 0.755 (RM252) with an average of 0.475 from 74 landraces from Tripura state in Northeast India.

The major allele frequency ranged from 0.06-0.54 with mean of 0.20. Meanwhile, the average gene diversity was 0.89 among 46 rice landraces with the range between 0.64-0.97. Similar gene diversity results were also reported in indigenous rice (*Oryza sativa* L.) varieties in the Eastern Himalayan region of Northeast India by Choudhury *et al*., 2013 (0.6188 to 0.7908) and in 24 rice genotypes by Furkanur *et al*., 2015 (0.419-0.884).

TABLE III

Data on number of alleles, number of polymorphic alleles, polymorphism per cent, PIC value, major allele frequency and gene diversity (GD) found among 46 rice landraces for 73 SSR markers

Sl. No.	Primers	Number of alleles	Number of polymorphic alleles	Polymorphic Bands (%)	PIC Value	Major allele frequency	Gene Diversity
1	RM1013	9	9	100	0.81	0.27	0.81
2	RM 1328	7	7	100	0.75	0.27	0.78
3	RM 1817	12	12	100	0.90	0.13	0.91
4	RM 1026	6	6	100	0.67	0.40	0.72
5	RM 6308	8	8	100	0.76	0.37	0.79
6	RM 7102	20	20	100	0.86	0.32	0.87
7	RM 7000	6	6	100	0.69	0.40	0.73
8	RM 6018	5	5	100	0.64	0.42	0.70
9	RM 6022	15	15	100	0.90	0.15	0.91
10	RM 6404	13	13	100	0.84	0.23	0.86
11	RM 1161	17	17	100	0.91	0.15	0.91
12	RM 6775	14	14	100	0.84	0.27	0.86
13	RM 6306	10	10	100	0.82	0.21	0.84
14	RM 7424	13	13	100	0.81	0.33	0.82
15	RM 6673	29	29	100	0.95	0.09	0.95
16	RM 1099	15	15	100	0.86	0.23	0.87
17	RM 3249	34	34	100	0.96	0.06	0.96
18	RM 5753	24	24	100	0.93	0.13	0.94
19	RM 1159	18	18	100	0.93	0.10	0.93
20	RM 2136	19	19	100	0.93	0.13	0.93
21	RM 2190	34	34	100	0.96	0.07	0.96
22	RM 8231	20	20	100	0.91	0.19	0.92
23	RM 3529	21	21	100	0.91	0.15	0.92
24	RM 3646	27	27	100	0.94	0.15	0.94
25	RM 7389	29	29	100	0.93	0.19	0.93
26	RM 7642	22	22	100	0.94	0.10	0.94
27	RM 3563	18	18	100	0.93	0.10	0.93
28	RM 8213	35	35	100	0.96	0.06	0.97
29	RM 6818	29	29	100	0.93	0.17	0.94
30	RM 84	18	18	100	0.92	0.15	0.92
31	RM 14	9	9	100	0.84	0.19	0.86
32	RM 3431	33	33	100	0.96	0.08	0.96
33	RM 10	14	14	100	0.87	0.25	0.88
34	RM 22	21	21	100	0.93	0.13	0.93
35	RM 18	13	13	100	0.84	0.23	0.85
36	RM 23	26	26	100	0.92	0.19	0.93
37	RM 5095	13	13	100	0.84	0.23	0.86

Sl. No.	Primers	Number of alleles	Number of polymorphic alleles	Polymorphic Bands (%)	PIC Value	Major allele frequency	Gene Diversity
38	RM 5183	22	22	100	0.92	0.15	0.93
39	RM 3525	16	16	100	0.87	0.25	0.88
40	RM 3486	19	19	100	0.92	0.13	0.93
41	RM166	19	19	100	0.93	0.10	0.93
42	RM 240	17	17	100	0.91	0.15	0.91
43	RM 223	23	23	100	0.93	0.13	0.94
44	RM 224	11	11	100	0.85	0.21	0.86
45	RM 13	21	21	100	0.93	0.17	0.93
46	RM 208	10	10	100	0.77	0.40	0.79
47	RM 319	17	17	100	0.88	0.21	0.89
48	RM 263	4	4	100	0.59	0.52	0.64
49	RM 178	22	22	100	0.92	0.17	0.92
50	RM 141	12	12	100	0.85	0.21	0.87
51	RM 163	8	8	100	0.63	0.54	0.66
52	RM 154	14	14	100	0.85	0.29	0.86
53	RM 138	22	22	100	0.92	0.15	0.93
54	RM 519	16	16	100	0.87	0.25	0.88
55	RM 526	19	19	100	0.92	0.13	0.93
56	RM151	22	22	100	0.93	0.13	0.93
57	RM 1003	18	18	100	0.91	0.15	0.92
58	RM 302	25	25	100	0.94	0.11	0.94
59	RM 3825	26	26	100	0.94	0.09	0.95
60	RM 5552	16	16	100	0.92	0.11	0.92
61	RM 246	17	17	100	0.91	0.13	0.92
62	RM 3542	21	21	100	0.93	0.10	0.94
63	RM 6838	12	12	100	0.86	0.26	0.87
64	RM 72	21	21	100	0.92	0.13	0.93
65	RM 1896	21	21	100	0.93	0.10	0.93
66	RM 206	21	21	100	0.93	0.13	0.93
67	RM 4862	16	16	100	0.89	0.19	0.90
68	RM 7102	22	22	100	0.92	0.15	0.93
69	RM 1337	16	16	100	0.88	0.21	0.89
70	RM 155	11	11	100	0.85	0.21	0.87
71	TRS 33	17	17	100	0.88	0.21	0.89
72	JJ 133-T3	14	14	100	0.83	0.31	0.84
73	NMSM	17	17	100	0.87	0.19	0.88
	Mean	18	18	100	0.88	0.20	0.89
	Total	1301.00	1301.00	7300.00	64.00	14.25	64.76

Genetic distance among accessions

The dendrogram constructed based on SSR markers differentiated 46 landraces, which were distinctly clustered into three main clusters A, B and C (Fig. 3 and Fig. 4). Cluster A comprised of 26

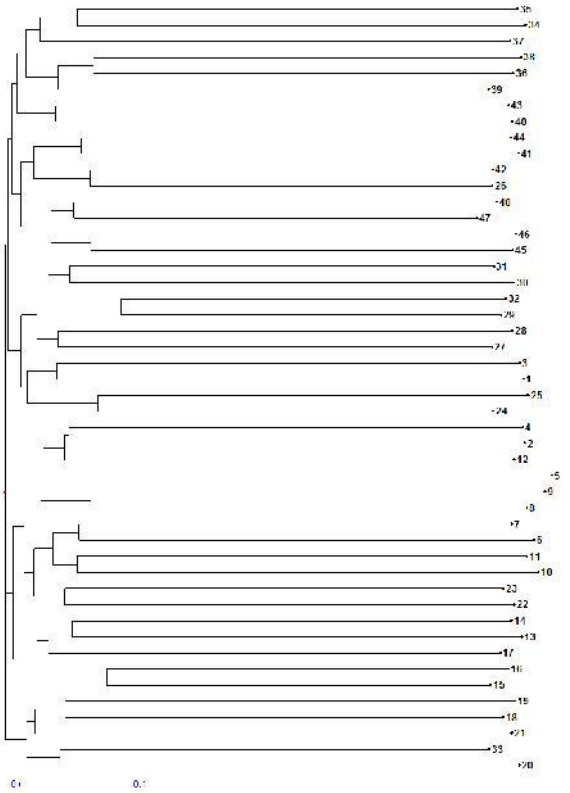

Fig. 3 : Genetic similarity among 46 rice genotypes based on simple sequence repeat (SSR) marker data.

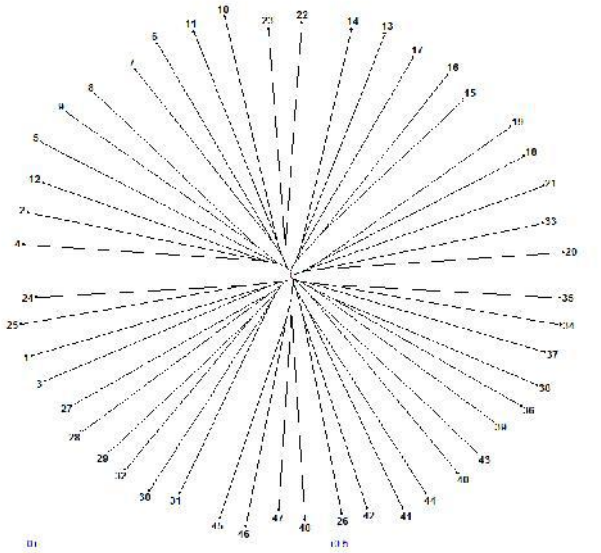

Fig. 4 : Neighbour- joining tree results based on Darwin version 5.0 showing genetic relationships among 46 rice landraces.

landraces and was further subdivided into two sub clusters A_1, which contains sixteen genotypes and A_2, which contains ten genotypes. This group consists of more than fifty per cent of the 46 landraces used in the current study. Similarly, 17 landraces were placed in cluster B and this cluster was further subdivided into two sub clusters B_1 which contains twelve genotypes and B_1 with 5 landraces. Cluster C comprised of five landraces, which is the smallest cluster (Table IV). The dendrogram revealed that the

TABLE IV

Grouping of rice landraces into different clusters on the basis of SSR data

Clusters	Sub-cluster	Number of genotypes	Landraces
A	A1	16	Naga Batta, Neergula Batta, Parimala Sanna, Rat Bath, Neergoli, Raja Bhog, Sugandhi, Sannaki, Sampige Gidda, Selam Sanna, Sampige Batta, Karigaj, MAS-946, MAS-26, Uduru Mallige, Sirisi Local
	A2	10	Matakka Ra, Mugad Sugandha, Jiddu Batta, Mallige, Mysore Mallige, Kari Kandaga, Bilidoddi Marthylaya, Bangara Sanna, Kirwana, Krishna Leela
B	B1	12	Bangara Kaddi, Bilidoddi Buddha, Honne Kattu, Chithaga, Gidda Batta, Giddaralia Kamala, Gamnada Batta, Dodda Mulare, Halu Gidda, Halubulla, Kari Batta, Kempu dodigidda
	B2	5	JenuGudu, Jeerige Salaiah, Kysare, Kari Doddi, Kotambari Nelu
C		5	Kalikatesi, Kaduvelpe, Kadulite, Muttina Sanna, Kappu Batta

genotypes which were derivatives of genetically similar type clustered together into same group. MAS-26 and MAS -946 both are aerobic rice used as control in present study and fall in same cluster A1. Variation in the DNA sequence and its associated influence on morphological traits could be the possible underlying reason for distinctness and diversity of an individuals and their unique clustering in the present diversity study. Salgotra *et al*., 2015 reported that 141 basmati rice were grouped into four major clusters and the genotypes were well clustered based on their place of collection and geographical region. Likewise, neighbor-joining tree of 249 Chile rice accession based on Jaccard's coefficient grouped accessions into two main groups, the temperate *japonica* (243 genotypes) and the non-*japonica* type (6 genotypes) and most of the 249 genotypes analyzed were distinguished by the 30 SSRs (Becerra *et al* 2017). These results clearly shows that genotypes falls in same cluster when their characters were tightly linked and genotypes fall in different clusters due to their evolutionary changes operating at DNA level and its consequence on the adaptability of the individual in different agro morphological conditions and domestication.

Genetic diversity resultsreported herein shows that rice landraces from different part of Karnataka consists of genetically diverse population and SSR markers were effectively employed to assess the genetic relationship between rice landraces. Diversity parameters can be used in crop improvement to establish a core collection as part of the germplasm collection management and to sample maximum of genetic variation. The study revealed that rice landraces from different part of Karnataka constitute three major genetic clusters as per Power Marker software and four major cluster as per neighbour-joining tree by using Darwin software.

Thus, present study revealed wide variation among the rice landraces and SSR markers exhibited 100 per cent polymorphism. The result indicated that the information about the genetic diversity will be very useful for identification of landraces, selection of appropriate parents for breeding programs and germplasm conservation.

REFERENCES

ANUPAM, A., IMAM, J., QUATADAH, S. M., SIDDAIAH, A., DAS, S. P., VARIAR M. AND MANDAL, N. P., 2017, Genetic diversity analysis of rice germplasm in Tripura state of Northeast India using drought and blast linked markers. *Rice Sci.,* **24** : 10 - 20.

BABU, B. K., MEENA,V., AGARWAL, V. AND AGRAWAL, P. K., 2014, Population structure and genetic diversity analysis of Indian and exotic rice (*Oryza sativa* L.) accessions using SSR markers. *Mol. Biol. Rep.,* **41 :** 4328 - 39.

BECERRA, V., PAREDES, M., FERREIRA, M. E., GUTIÉRREZ, E. AND DÍAZ, L. M., 2017, Assessment of the genetic diversity and population structure in temperate *japonica* rice germplasm used in breeding in Chile, with SSR markers. *Chilean J. Agric. Res.,* **77** : 15 - 26.

BHUYAN, N., BORAH, B. K. AND SHARMA, R. N., 2007, Genetic diversity analysis in traditional lowland rice (*Oryza sativa* L.) of Assam using RAPD and ISSR markers. *Curr. Sci.,* **93 :** 967 - 972.

BORBA, T. C. O., BRONDANI, R. P. V., RANGEL, P. H. N. AND BRONDANI, C., 2009, Microsatellite marker-mediated analysis of the EMBRAPA rice core collection genetic diversity. *Genetica,* **137 :** 293 - 304.

BOSTEIN, D., WHITE, R. L., SKOLINCK, M. AND DAVIS, R.W., 1980, Construction of a genetic linkage mapping restriction fragment length polymorphism. *Am. J. Human Gen.,* **32** : 314 - 313.

CHAKHONKAEN, S., PITNJAM, K., SAISUK, W., UKOSKIT, K. AND MUANGPROM, A., 2012, Genetic structure of thai rice and rice accessions obtained from the International Rice Research Institute. *Rice,* **5 :** 19.

CHOUDHURY, B., KHAN, M. L. AND DAYANANDAN, S., 2013, Genetic structure and diversity of indigenous rice (*Oryza sativa* L.) varieties in the Eastern Himalayan region of Northeast India. *Springer Plus,* **2 :** 228.

DOYLE, J. J. AND DOYLE, J. L., 1987, A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.,* **19 :** 11 - 15.

ETEMAD, A., MAZIAH, M. AND DAUD, S. K., 2012, Determination of genetic relatedness among selected rice (*Oryza sativa* L.) cultivars using microsatellite markers. *African J. Biot.,* **11 :** 7158 - 7165.

FURKANUR, R. M., RAHMAN, M. M., HALDER, J., REHANA, S., BEGUM, S. AND ISLAM, M. M., 2015, Genetic diversity and SSR marker assisted salt screening of rice (*Oryza sativa* L.). *Biosci. Bioeng. Commun*., **1** : 29 - 37.

GRACIA, A. A. F., BENCHIMOL, L. L., ANTONICA, M. M., GERALDI 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.

HITTALMANI, S., KAHANI, F., DHANAGOND, S. M., AND RAO, A. M., 2013, DNA marker characterization for allele mining of blast and bacterial leaf blight resistant genes and evaluation for grain yield, **12 :** 2331 - 2340.

HOSSAIN, M. Z., RASUL, M. G., ALI, M. S., IFTEKHARUDDAULA, K. M. AND MIAN MA, 2007, Molecular characterization and genetic diversity in fine grain and aromatic landraces of rice using microsatellite markers. *Bangladesh J. Genet. Pl. Breed.*, **20:** 1 - 10.

JAIN, S., JAIN, R. K. AND MC COUCH., S. R., 2004, Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labelled microsatellite markers. *Theor. Appl. Genet.*, **109 :** 965 - 977.

JOSHI, H. J. AND BEHERA, R. K., 2006, Identification and differentiation of indigenous non-basmati aromatic rice genotypes of India using microsatellite markers. *African J. Biot.*, **6 :** 348 - 354.

KOBAYASHI, A., EBANA, K., FUKUOKA, S. AND NAGAMINE, T., 2006, Microsatellite markers revealed the genetic diversity of an Old Japanese Rice Landrace 'Echizen'. *Genetic Resources and Crop Evolution* **53 :** 499 - 506.

LIU, K. AND MUSE, S. V., 2005, Power marker : An integrated analysis environment for genetic marker analysis. *Bioinform.*, **21** : 2128 - 2129.

MICHAEL, J. T., NICHOLAS, R. P., JOKO, P. K. R., TRIJATMIKO, TIUR, S. S. AND MC COUCH, S. R., 2009, Genetic diversity of isolated populations of Indonesian landraces of Rice (*Oryza sativa* L.) collected in east Kalimantan on the Island of Borneo. *Rice*, **2:** 80 - 92.

NAGARAJU, J., KATHIRVEL, M., KUMAR, R. R., SIDDIQ, E. A., AND HASNAIN, S. E., 2002, Genetic analysis of traditional and evolved Basmati and Non-Basmati rice varieties by using fluorescence based ISSR, PCR and SSR markers. *Proc. Natl. Acad. Sci.*, **99 :** 5836 - 5841.

NEI, J., PETER, M. AND MACKILL, B. J., 2002, Evaluation of genetic diversity in rice sub species using microsatellite markers. *Crop Sci.*, **42 :** 601 - 607.

PERRIER, X. AND COLLET, J. P., 2006, Darwin software (http://darwin. cirad.fr/).

SALGOTRA, R. K., GUPTA, B. B., BHAT, J. A. AND SHARMA, S., 2015, Genetic diversity and population structure of basmati rice (*Oryza sativa* L.) germplasm collected from North Western Himalayas using trait linked SSR markers. *PLoS One* **10 :** 1 - 19.

SHIVAPRIYA, M. AND HITTALMANI, S. (2006), Detection of genotype specific fingerprints and molecular diversity of selected Indian locals and landraces of rice (*Oryza sativa* L.) using DNA markers. **66** : 1 - 5.

SINGH, N., CHOUDHURY, D. R., SINGH, A. K., KUMAR, S. AND SRINIVASAN, K., 2013, Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of indian rice varieties. *PLoS ONE*, **8 :** 1 - 12.

SONG, Z. P., XUX WANG, B., CHEN, J. K. AND LU B. R., 2003, Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. *Theor. Appl. Genet.*, **107 :** 1492 - 1499.

TANG, S. X., JIANG, Y. Z., WEI X. H., LI Z. C. AND YU, H. Y., 2002, Genetic diversity of isozymes of cultivated rice in china. *Acta Agron. Sin.*, **28 :** 203 - 207.

TEIXEIRA DA SILVA, J. A., 2005, Molecular markers for phylogeny, breeding and ecology in agriculture. *Genetic Resources and Biot.*, **3 :** 221 - 256.

VIRK, P. S., NEWBURY, J. H., BRYAN, G. J., JACKSON, M. T. AND FORD-LLOYD, B.V., 2000, Are mapped or anonymous markers more useful for assessing genetic diversity? *Theor. Appl. Genet.*, **100 :** 607 - 613.

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