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Analysis of Unique Rice (*Oryza sativa* L.) Germplasm Accessions Using ISSR, SSR and Morphological Markers

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Rice is a major crop grown under all the conditions due to its adaptive nature. Present investigation of genetic diversity analysis was based on 46 selected unique germplasm (based on their morphological and biochemical properties) from IGKV, Raipur and 2 check varieties of rice. Diversity analysis was conducted using ISSR, microsatellite markers and 14 agro-morphological traits. The study revealed that the average polymorphic information content (PIC) value with ISSR markers was 0.85, which ranged from 0.79 (UBC 808) to 0.88 (UBC818). The SSR markers yielded mean PIC value of 0.49, ranging from 0.12 (OSR 13) to 0.74 (RM 1 and RM 20). The similarity coefficient of major clusters ranged from 0.48 to 0.88 (SSR and ISSR) and 0.46 to 0.99 (morphological). Among the 48 unique germplasm, the diversity analysis based on morphological characters showed that *Anterved Jal Ponga*, *Nariyal Phool* and *Ganga Baru* within cluster A were most similar with a similarity coefficient of 0.999. With SSR and ISSR diversity analysis, *Kapoor Sar* and *Chini Kapoor* were most similar with a similarity coefficient of 0.875. *Do Dana Bajrang Bali*, *Chepti Gurmatiya* and *Gathuwan*, *Badshah Bhog* and *Ganga Baru* were highly diverse indicating that these accessions can be used in future breeding program.

Key Words: Genetic diversity, ISSR, Morphological markers, Rice germplasm, SSR

Introduction

Rice is the important crop grown under different environmental conditions. Possibly the oldest domesticated grain (~10,000 years), rice is the staple food for 3 billion people (60% of the world's population) and is the largest single use of land for producing food, covering 9% of the earth's arable land (Nanda, 2000). Rice, a member of the family Poaceae, originated from South-East Asia, where more than 90% of world's rice is produced and consumed (Li and Xu, 2007), making it immensely important for food security of Asia. Out of 24 species of rice, only two species (*O. glabberima* and *O. sativa*) are cultivated. Variation within *O. sativa* is extensive, due to its adaption to a wide range of geographical and ecological niches and climatic regimes.

The understanding and knowledge of genetic variation and genetic similarities present within individuals or populations are useful for the efficient use of genetic resources in a breeding program. There are a number of markers available and used for diversity analysis like RFLP, AFLP, ISSR, SSR, RAPD and morphological *etc*. The majority of simple sequence repeats (SSR) that are present in high or intermediate frequencies in landraces ultimately survive into modern elite cultivars and hybrids (Singh *et al*., 2011). The high resolution power and efficient production of massive amount of SSR data is very useful for genetic diversity analysis and evolutionary studies of crop plants (Yang *et al*., 1994; Struss and Plieske, 1998; Tenzer and Gessler, 1999; Singh *et al*., 2011). The purpose of this study was to analyze the genetic diversity of selected germplasm to evaluate the potential usefulness of germplasm for development of better variety.

Materials and Methods

A total of 46 germplasm accessions from IGKV, Raipur and two check varieties (MTU 1010 and *Mahamaya*) of rice were taken for diversity analysis (Table 1). The germplasm lines represented a care sample of 23,250 accessions held at IGKV, Raipur. Fourteen morphological trait were recorded in 1 to 9 scale (Standard Evaluation System, IRRI) under normal transplanted field condition during *Kharif* 2011 at Research cum Instructional Farm, IGKV, Raipur ($21^0 16'$N and $81^0 36'$E at altitude of 289.6 masl). The morphological characters *viz*., coleoptyle color, early plant vigour, basal leaf sheath color, leaf blade color, leaf pubescence, flag leaf angle, ligule color, ligule shape, collar color, auricle color, stigma color, awning, hull color, seed coat color (kernel) were recorded.

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Table 1. Detail of germplasm used in diversity analysis

S.No.	Name of Germplasm	Accession No.	IC No.	Block	District	State
1	Kadam Phool	K:2382	IC390768	Bijapur	Bijapur	Chhattisgarh
2	Koudi Dhull	K:1849	IC390770	Dharamjaigarh	Raigarh	Chhattisgarh
3	Nariyal Phool	N:796	IC390772	Sarai pali	Mahasamund	Chhattisgarh
4	Do Dana	D:612	IC390777	Bilaspur	Bilaspur	Chhattisgarh
5	Pankhi	P:189	IC390778	Deobhog	Gariyaband	Chhattisgarh
6	Tulsi Manjari	BD:1290	IC390784	Sabour	Bihar	Bihar
7	Badshah Bhog	B:42	NA	Bakwand	Bastar	Chhattisgarh
8	Kari Gilas	K:760	NA	Charama	Kanker	Chhattisgarh
9	Parmal	P:445	NA	Paraswara	Balaghat	Madhya Pradesh
10	Roti	R:299	IC390790	Bastar	Bastar	Chhattisgarh
11	Layacha	L:114	NA	Dongargaon	Durg	Chhattisgarh
12	Hardi Gathi	H:17	NA	Darma	Bastar	Chhattisgarh
13	Bajrang Bali	B:640	IC390796	Bhatapara	Baloda bazar	Chhattisgarh
14	Bokra Mundi	B:2318	IC390797	Deobhog	Gariyaband	Chhattisgarh
15	Kapoor Sar	BD:1364	IC390798	Chhuriya	Rajnandgaon	Chhattisgarh
16	Chini Kapoor	C:459	IC390800	Sarona	Sukma	Chhattisgarh
17	Kali Kamod	BD:680	IC390802	Arang	Raipur	Chhattisgarh
18	Chhatri	BD:444	IC390803	Darba	Gwalior	Madhya Pradesh
19	Atma Shital	BD:293	IC390804	Chhindgarh	Sukma	Chhattisgarh
20	Jira Dhan	BD:575	IC390806	Tilda	Raipur	Chhattisgarh
21	Chinnor	BD:1295	IC390808	Tilda	Raipur	Chhattisgarh
22	Ganga Baru	BD:291	IC390811	Chhindgarh	Sukma	Chhattisgarh
23	Anterved	BD:956	IC390814	Jabera	Damoh	Madhya Pradesh
24	Kubri Mohar	BD:401	IC390816	Magarlod	Dhamtari	Chhattisgarh
25	Chepti Gurmatiya	C:843	IC390817	Chhui khadan	Rajnandgaon	Chhattisgarh
26	Farsa Phool	F:16	IC390821	Koili beda	Kanker	Chhattisgarh
27	Hanuman Langur	H:470	IC390822	Mahasamund	Mahasamund	Chhattisgarh
28	Jal Ponga	J:311	IC390826	Baihar	Balaghat	Madhya Pradesh
29	Suldhan	S:1470	IC390829	Deobhog	Gariyaband	Chhattisgarh
30	Loktimachhi	L:1250	NA	Darma	Bastar	Chhattisgarh
31	Jou Phool	J:333	IC390837	Lailunga	Raigarh	Chhattisgarh
32	Lahsun Bhog	L:985	IC390838	Kunkuri	Jashpur	Chhattisgarh
33	Jhilli	J:384	IC390840	Patthalgaon	Jashpur	Chhattisgarh
34	Shri Kamal	S:663	IC390842	Raipur naikin	Sidhi	Madhya Pradesh
35	Son Banko	S:167	IC390843	Baloda bazar	Baloda bazar	Chhattisgarh
36	Urai Butta	U:9	NA	Kundam	Jabalpur	Madhya Pradesh
37	Safri Deshi	S:99	NA	Masturi	Bilaspur	Chhattisgarh
38	Gathuwan	G:1039	IC390847	Abhanpur	Raipur	Chhattisgarh
39	Maharaji	M:504	IC390850	Ghughari	Mandla	Madhya Pradesh
40	Danwar	D:1363	IC390852	Sarona	Bastar	Chhattisgarh
41	Baisur	B:1611	IC390853	Dongargarh	Rajnandgaon	Chhattisgarh
42	Shital Bhog	S:1684	IC390856	Kunkuri	Jashpur	Chhattisgarh

Contd.....

Table 1. Contd.....

S.No.	Name of Germplasm	Accession No.	IC No.	Block	District	State
43	Vishnu Bhog	V:28	IC390859	Badraf nagar	Balrampur	Chhattisgarh
44	Jal Keshar	J:117	IC390869	Sihore	Jabalpur	Madhya Pradesh
45	Rani Kajar	R:123	IC390873	Tilda	Raipur	Chhattisgarh
46	Nariyal Chudi	N:174	IC390876	Koyalibeda	Kanker	Chhattisgarh
47	MTU 1010*	IET 15644	Krishnaveni × IR 64	Pedigree selection	Maruteru	Andhra Pradesh
48	Mahamaya*	R 320-298 IET 10749	Asha × Kranti	Pedigree selection	IGKV, Raipur	Chhattisgarh

NA= Not Available, * check variety

Molecular studies were carried out at Plant Molecular Biology Laboratory, Department of Genetics and Plant Breeding, IGKV, Raipur, during 2012. DNA was extracted from fresh leaf using MiniPrep method (Doyle and Doyle, 1987). The DNA samples were analyzed using 109 SSR and 14 ISSR primers. PCR amplification for SSR was performed in a total volume of 20 µl and the reaction mixture contained 10 X Assay buffer, 1 mM dNTP mix, 5 pM forward and reverse primers, 40 ηg of template DNA and 1 unit Taq polymerase. Reaction mixture was amplified using 96-well Veriti Applied Biosystems thermal cycler, USA. After an initial denaturation step of 95^0C for 5 min, the amplification was carried out for 34 cycles comprising 1 min each of 94^0C of denaturation, 55^0C of annealing and 72^0C of extension. The final elongation step was extended to 7 min at 72^0C followed by storage at 4^0C. After the PCR reaction was completed, 5 µl of 6 X loading dye was added to PCR reaction and 7 µl (PCR reaction with dye) was loaded on 5 % PAGE in a vertical gel electrophoresis system (CBS scientific, model MGV-202-33, USA) with 180V for 1.5 hours. Gel was then stained with ethidium bromide and DNA fragments were visualized with a UV transilluminator Bio-rad XR+ manufactured from USA (Fig. 1).

PCR amplification for ISSR was performed in a reaction volume of 25 µl containing 4 µl DNA (40 ng/µl), 2.5 µl 10X Buffer, 2 µl 1 mM dNTPs (dATP, dCTP, dTTP, dGTP), 1.75 µl 5 pM Primer, and 0.75 µl Taq polymerase (1 Unit). Amplification was carried out in a Veriti® thermal cycler (Applied Biosystems, USA) as follows: 1 cycle of 2 minutes at 95^0C, 35 cycles of 30 seconds at 95^0C, 35 cycles of 60 seconds at varying temperature, 35 cycles of 1.30 min at 72^0C, 1 cycle of 15 min at 72^0C and then stored at 4^0C. 4 µl BPB Dye/25 µl PCR reaction were mixed and loaded in 5 % PAGE gel and run at 120V for 2 h. After proper run gels were stained with ethidium bromide (EtBr) solution and gel images were recorded in Bio-Rad, Gel DOC unit manufactured from USA, under UV light (Fig. 2).

Specific amplification products were scored as present (1) or absent (0) depending on increasing order of their molecular weights of each DNA sample. Diversity analysis was done using NTSYSpc-2.02 software (Numerical Taxonomy System Biostatistics) and dendrogram was generated (Rohlf, 2000). The similarity matrix (SM) was generated with similarity coefficient for SSR, ISSR markers and morphological characters and cluster analysis was done using the SM similarity coefficient, with UPGMA (unweighted pair-group method with arithmetic averages) method. The polymorphism information content (PIC) value of SSR markers was calculated using the following formula (Anderson *et al*., 1993).

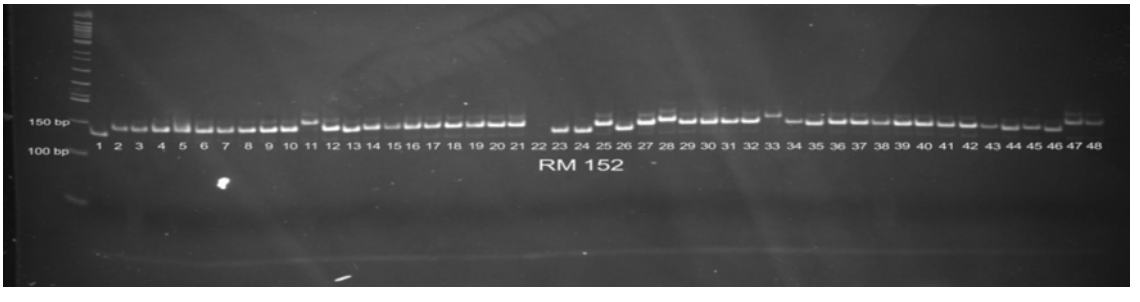

Fig. 1. SSR profile generated by microsatellite primer RM 152 of 48 germplasm (1-48 labels are as per the germplasm given in Table 1)

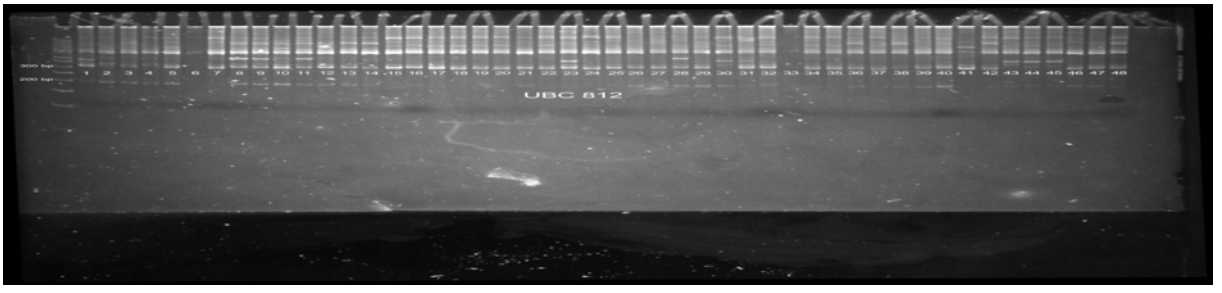

Fig. 2. ISSR profile of 48 germplasm generated by UBC 812 primer (1-48 labels are as per the germplasm given in Table 1)

$$PIC = 1 - \sum_{i=1}^{k} Pi^2$$

where, k is the total number of alleles (bands) detected for one SSR/ISSR locus and P_i is the frequency of the i^{th} allele (band) in all the samples analyzed.

Results and Discussion

Molecular diversity study using SSR and ISSR

Out of 109 SSR markers used in the study, 54 were polymorphic and produced a total of 160 alleles (Table 2). The number of alleles varied from 2 to 6 with an average of 2.96 polymorphic alleles per primer. Out of 54 primers 22, 19, 8, 3, and 2 primers showed 2, 3, 4, 5, and 6 alleles, respectively. RM 552 and RM 207 showed maximum 6 alleles, RM 341, RM 20 and RM showed 5 alleles. RM 224 and RM 208 showed similar allele and RM 1, RM 144, RM 25 and RM 242 showed dissimilar allele than earlier reported allele by Singh *et al.* (2011) for the same markers. The average PIC value was calculated 0.49 and which ranged from low of 0.12 (OSR 13) to a high of 0.74 (RM 1 and RM 20). Markers showing high PIC value are associated with higher level of polymorphism and therefore, more important.

Out of 14 ISSR primers used, 8 primers generated a total of 76 bands which were scored for determining the genetic relationship among genotypes (Table 3). UBC 812 showed maximum 12 bands and UBC 808 and UBC 810 showed minimum 7 bands. Average number of polymorphic bands per primer was 9.5. The average PIC value was calculated 0.85 and which ranged from low of 0.79 (UBC 808) to a high of 0.88 (UBC818).

Table 2. SSR markers used in diversity analysis

S.No.	Markers	Forward sequence (5' -------->3')	Reverse sequence (5' --------->3')	Ch #	No. of allele	PIC
1	RM 1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	1	5	0.74
2	RM 5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	1	3	0.64
3	RM 11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	7	3	0.55
4	RM 17	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA	12	3	0.48
5	RM 19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	12	3	0.61
6	RM 20	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	12	5	0.74
7	RM 25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC	8	3	0.59
8	RM 44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	8	2	0.50
9	RM 55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	3	2	0.50
10	RM 105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	9	2	0.47
11	RM 106	CGTCTTCATCATCGTCGCCCCG	GGCCCATCCCGTCGTGGATCTC	2	2	0.31
12	RM 116	TCACGCACAGCGTGCCGTTCTC	CAAGATCAAGCCATGAAAGGAGGG	11	2	0.49
13	RM 120	CACACAAGCCCTGTCTCACGACC	CGCTGCGTCATGAGTATGTA	11	2	0.49
14	RM 125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	7	3	0.49

Contd.....

Table 2. Contd.....

S.No.	Markers	Forward sequence (5' -------->3')	Reverse sequence (5' --------->3')	Ch #	No. of allele	PIC
15	RM 144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	11	4	0.70
16	RM 152	CCGTAGACCTTCTTGAAGTAG	GAAACCACCACACCTCACCG	8	3	0.29
17	RM 161	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG	5	2	0.46
18	RM 178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC	5	2	0.44
19	RM 207	CCATTCGTGAGAAGATCTGA	CACCTCATCCTCGTAACGCC	2	6	0.67
20	RM 208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	2	2	0.38
21	RM 214	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	7	4	0.36
22	RM 221	ACATGTCAGCATGCCACATC	TGCAAGAATCTGACCCGG	2	3	0.45
23	RM 222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG	10	3	0.53
24	RM 224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	11	2	0.46
25	RM 231	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	3	4	0.69
26	RM 234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	7	2	0.44
27	RM 242	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	9	2	0.33
28	RM 247	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	12	3	0.63
29	RM 248	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	7	4	0.67
30	RM 268	GTGCTATGCACGATCCATAGCA	CGTTTCTTTGGAAGCGGAGGGA	2	3	0.44
31	RM 277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	12	2	0.47
32	RM 278	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC	9	2	0.16
33	RM 281	ACCAAGCATCCAGTGACCAG	GTTCTTCATACAGTCCACATG	8	3	0.44
34	RM 298	CTGATCACTGGATCGATCATG	CATGCCAAGATGCAACAG	7	3	0.43
35	RM 341	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC	2	5	0.70
36	RM 348	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC	4	2	0.16
37	RM 403	GCTGTGCATGCAAGTTCATG	ATGGTCCTCATGTTCATGGC	1	4	0.64
38	RM 408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC	8	2	0.47
39	RM 411	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG	3	2	0.32
40	RM 433	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC	8	2	0.48
41	RM 444	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG	9	3	0.61
42	RM 447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC	8	4	0.52
43	RM 454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG	6	2	0.30
44	RM 474	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG	10	4	0.66
45	RM 484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC	10	3	0.23
46	RM 495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	1	3	0.47
47	RM 506	CGAGCTAACTTCCGTTCTGG	GCTACTTGGGTAGCTGACCG	8	2	0.49
48	RM 533	GCAACTGCTCTACGCCTCTC	CCTGAGGCTTCACCTACTCG	7	3	0.51
49	RM 536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC	11	2	0.35
50	RM 540	GCCTTCTGGCTCATTTATGC	CTAGGCCTGCCAGATTGAAC	6	3	0.45
51	RM 552	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC	11	6	0.72
52	RM 566	ACCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC	9	3	0.63
53	RM 3825	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG	1	4	0.70
54	OSR 13	CATTTGTGCGTCACGGAGTA	AGCCACAGCGCCCATCTCTC	3	2	0.12

The dendogram generated by UPGMA method indicated that all the 48 genotypes were grouped into two sub-clusters A and B. The major cluster A consisting of 42 genotypes and sub-clustering near the 0.490 similarity level. The other major cluster B consisted of 6 genotypes having 0.485 similarities coefficient with cluster A and sub clustering near the 0.505 similarity level (Fig. 3). The similarity coefficient ranged from 0.48 to 0.88. Germplasm *Kapoor Sar* and *Chini Kapoor* within cluster A were most similar with a similarity coefficient of 0.875 and germplasm *Badshah Bhog* and Ganga *Baru* were the least similar with similarity

Table 3. ISSR markers used in diversity analysis

S.No.	Markers	Primer sequence	No. of amplicons generated	TM (^{o}C)	Fragment size (bp)	No. of bands	PIC
1	UBC 807	AGAGAGAGAGAGAGAGT	17	53	300-1000	10	0.87
2	UBC 808	AGAGAGAGAGAGAGAGC	17	49	300-750	7	0.79
3	UBC 809	AGAGAGAGAGAGAGAGG	17	52	250-800	10	0.86
4	UBC 810	GAGAGAGAGAGAGAGAT	17	52-53	200-900	7	0.84
5	UBC 812	GAGAGAGAGAGAGAGAA	17	50	200-900	12	0.84
6	UBC 818	CACACACACACACACAG	17	52	250-1000	10	0.88
7	UBC 834	AGAGAGAGAGAGAGAGAT	18	50	380-850	11	0.85
8	UBC 836	AGAGAGAGAGAGAGAGGA	18	50	330-900	9	0.85

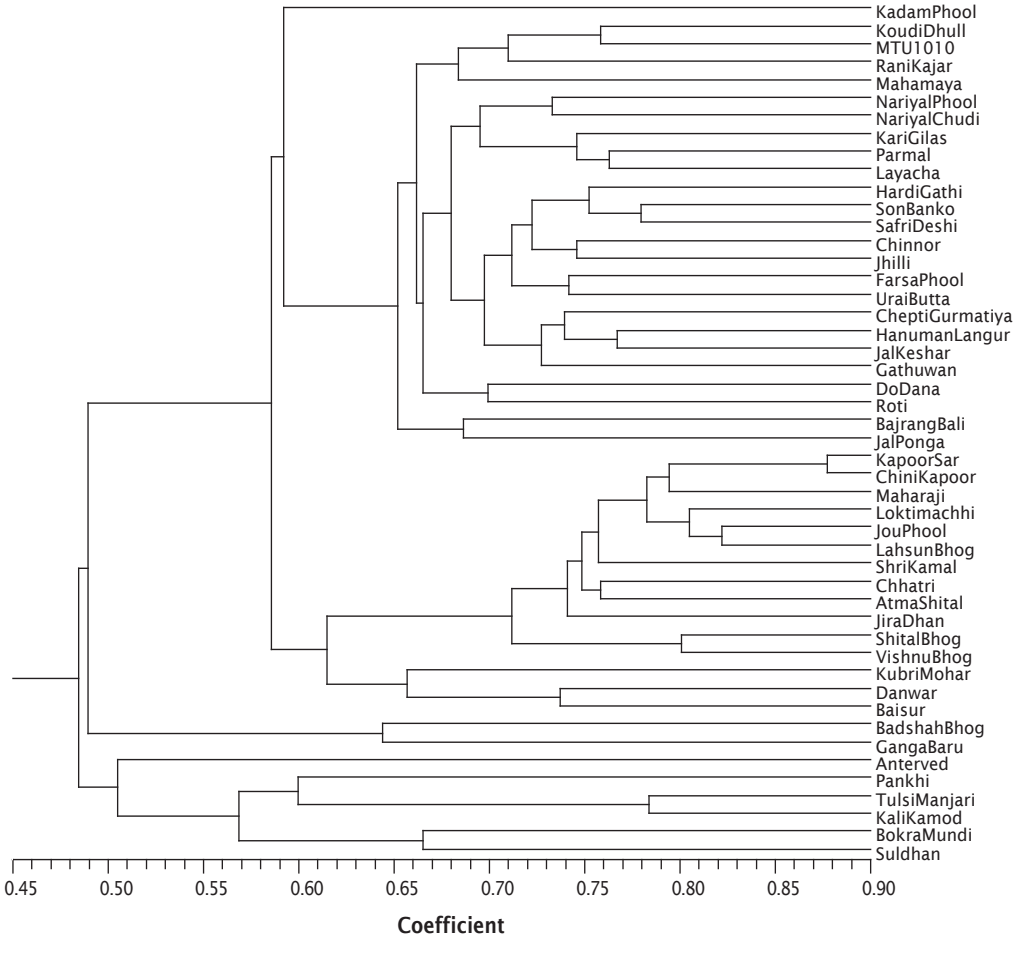

Fig. 3. Dendrogram constructed using UPGMA based on SM coefficient of 48 germplasm (SSR and ISSR markers) (A= Cluster A, B= Cluster B)

coefficient of 0.643. This study reveals the similarity and dis-similarity pattern between the germplasm accessions. The entries having high similarity shows that they have common genes in their ancestry whereas, the dissimilar ones clearly distinguish that these entries are of diverse nature (Thomson *et al*., 2007).

Morphological diversity

Fourteen morphological traits were recorded in 0-9 scale for diversity analysis. Similarity coefficient ranged from 0.460 to 0.999 for morphological based diversity. The dendogram indicates that there was a major cluster A consisting of 40 genotypes, showing sub clustering near the 0.518 similarity level. The other major cluster B consisted of 8 genotypes having 0.485 similarities coefficient with cluster A and clustering near 0.512 similarity level (Fig. 4). Germplasm *Anterved* and *Jal Ponga*, *Nariyal Phool* and *Ganga Baru* within cluster A were most similar with a similarity coefficient of 0.999 and germplasm *Do Dana* and *Bajrang Bali*, *Chepti Gurmatiya* and *Gathuwan* within cluster B were the least similar with similarity coefficient of 0.692.

The comparative analysis of SSR, ISSR and agro-morphological markers indicates that *Kadam Phool*, *Jal Ponga*, *Koudi Dhull*, *Hardi Gathi*, *Nariyal Phool*, *Ganga Baru*, *Kapoor Sar*, *Jouphool*, *Lahsun Bhog*, *Layacha*, *Hanuman Langur*, *Tulsi Prasad*, *Safri Desi*, *Chini Kapoor*, *Kubri Mohar*, *Jira Dhan*, *Sonbanko*, *Chinnor*, *Jhilli*, *Loktimachhi*, *Nariyal Chudi*, *Atmashital*, *Maharaji*, MTU1010, *Vishu Bhog*, *Rani Kajar*, *Badsah Bhog*, *Shital Bhog*, *Baisur*, *Chhatri*, *Mahamaya*, *Shri Kamal*, *Parmal*, *Farsa Phool* and *Roti* were common in cluster A using all the markers mentioned above.

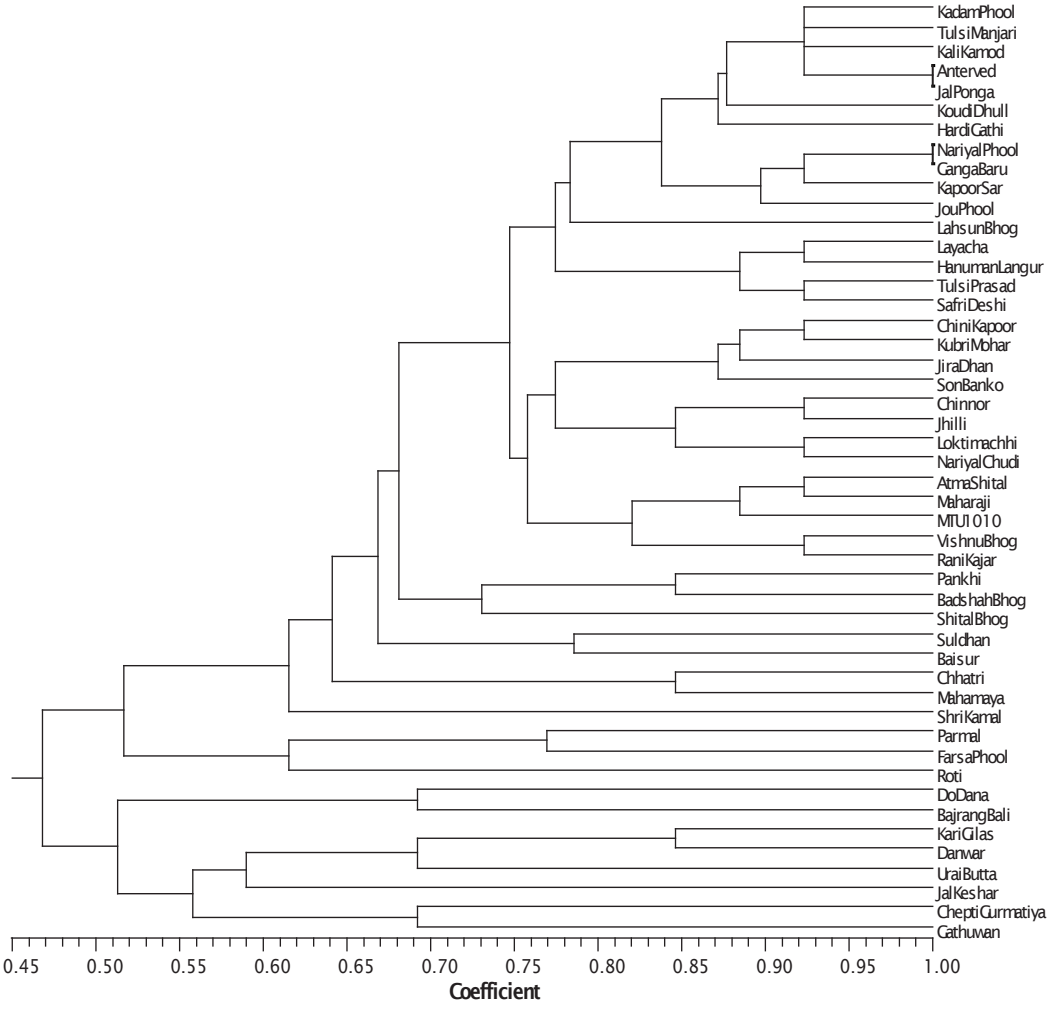

Fig. 4. Dendrogram constructed using UPGMA based on SM coefficient of 48 germplasm (Morphological markers) (A= Cluster A, B= Cluster B)

Conclusions

The local germplasm of rice used in the present study showed a lot of potential for various traits and hence, could be used for further improvement for incorporating certain important and valuable traits. Based on diversity analysis germplasm, *Do Dana* and *Bajrang Bali*, *Chepti Gurmatiya* and *Gathuwan*, *Badshah Bhog* and *Ganga Baru* were least similar so that these combination can be further utilized for developing better varieties.

References

Anderson JA, GA Churchill, JE Sutrique, SD Tanksley and ME Sorrells (1993) Optimizing parental selection for genetic linkage maps. *Genome* **36**: 181-186.

Doyle JJ and JL Doyle (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phyto. Chem. Bull*. **19**: 11-15.

Li ZK and JL Xu (2007) Breeding for drought and salt tolerant rice (*Oryza sativa* L.): progress and perspectives. In: MA Jenks *et.al* (ed.) *Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops*. Springer, USA, pp 531-564.

Nanda JS (2000). *Rice Breeding and Genetics*. *Research Priorities and Challenges*. Oxford & IBH Publishing Co. Pvt., New Delhi, pp 1-15.

Rohlf FJ (2000) NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.10e. Exeter Publications, New York.

Singh B, SP Singh and J Kumar (2011) Assessment of genetic diversity in aromatic rice (*Oryza sativa* L.) using morphological, physicochemical and SSR markers. *Indian J. Genet*. **71**: 214-222.

Struss D and J Plieske (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. *Theor. Appl. Genet*. **97**: 308-315.

Tenzer I and C Gessler (1999) Genetic diversity of *Venturia inaequalis* across Europe. *Eur. J. Plant Pathol*. **105**: 545-552.

Thomson MJ, EM Septiningsih, F Suwardjo, TJ Santoso, TS Silitonga and SR McCouch (2007) Genetic diversity analysis of traditional and improved Indonesian rice (*O. sativa* L.) germplasm using microsatellite markers. *Theor. Appl. Genet*. **114**: 559-568.

Yang GP, MA Sahgai Maroof, CG Xu, Q Zhang and RM Biyashev (1994) Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Mol. Gene Genet*. **245**: 187-194.