Emir. J. Food Agric. 2014. 26 (6): 497-507
doi: 10.9755/ejfa.v26i6.15722
http://www.ejfa.info/

Regular Article

PLANT MOLECULAR BIOLOGY

Genetic diversity analysis of colored and white rice genotypes using Microsatellite (SSR) and Insertion-Deletion (INDEL) markers

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Abstract

Genetic diversity analysis of 19 coloured and white rice genotypes were conducted using 14 Simple Sequence Repeat (SSR) and 21 Insertion Deletion (INDEL) markers. Among them, polymorphic results were obtained for 9 SSR and 12 INDEL markers. The PIC values ranged from 0.36(RM484) to 0.78 (RM 167) and 0.50 (R9M20) and 0.81 (R9M10) for SSR and INDEL markers respectively. In the case of both SSR and INDEL markers highest genetic diversity was observed between Krishna Kamod (White pericarp) and IRST 1 (Red pericarp) whereas least genetic diversity was observed between Lal Kada (Red pericarp) and Krishna Kamod (White pericarp). However, it was also found that brown, black and red pericarp share more similarity among themselves. It was also observed that INDEL markers reveals greater diversity among the genotypes as compared to SSR markers which was indent from the low average similarity index observed in the former. On the whole INDEL markers were found to be more efficient than SSR markers for diversity analysis.

Key words: Red rice, SSR, INDEL, Polymorphism, Heterozygosity

Introduction

Rice is the second most important cereal crop in the world. Among the rice growing countries in the world, India has the second largest area under rice crop (about 45 million ha) and ranks second in production next to China. In Asia, traditionally, rice with varied colours such as red, purple, brown yellow and green have been grown. Coloured rice has been preferred in the past for their special features in medicinal value and exclusive taste as compared to common rice. Coloured rice have higher concentrations of protein, total essential amino acids, vitamin B1 and minerals (Suzuki et al., 2004; Yoshida et al., 2010). In India, coloured rice is prevalent in pockets of South, North western and North eastern Himalayan region (Deepa et al., 2008). The red rice was preferred by people in many parts of India, Sri-Lanka and Bhutan. Two loci have been identified using classical genetic analysis for red pericarp coloration, *Rc* (brown pericarp and seed coat) and *Rd* (red pericarp and seed coat). When present together these loci produce red seed colour whereas *Rd* in the absence of *Rc* provides brown seeds whereas *Rc* alone has no phenotype (Kato and Lshikawa, 1921). A mutation in the *Rc* gene that changed the red seed of wild rice into the white seeds of modern rice is shared by a large majority of rice varieties, regardless of subspecies (Megan et al., 2007). The study of morpho-agronomic variability is the classical way of assessing genetic diversity for plant breeders. Genetic marker screening is based on the survey of genetic diversity as revealed by variation at specific gene loci and provides information about the amount and distribution of genetic diversity within and among populations (Buu and Lang., 1999). A wide variety of DNA markers such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Insertion/Deletion markers (INDEL) etc. have been extensively used in rice for genetic diversity analysis, phylogenetic and evolutionary studies, mapping and tagging genes for quantitative traits of agronomic importance and Marker Assisted Selection(MAS). Molecular markers like

Received 30 June 2013; Revised 18 February 2014; Accepted 22 February 2014; Published Online 25 March 2014

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RAPD, AFLP are extensively been used to quantify the inter-specific, intra-specific and inter-generic variability in different plant groups and crop varieties (Pawar et al., 2013). Kibria et al. (2009) has screened several rice varieties for studying the genetic diversity by using SSR and RAPD markers.

SSR markers can estimate genetic diversity between cultivars e.g. between parents of genepool or between plants extracted from a population or between populations. Microsatellites are more powerful for the identification of within cultivar variation (Lapitan et al., 2007). Zhou et al. (2003) investigated the genetic diversity and genetic structure of natural populations of *O. rufipogon* in China using SSR markers and information was found to be significant. Steele et al. (2008) reported that a genome wide rice polymorphism database developed by Shen et al. (2004) contains more than 4,00,000 insertion/ deletion polymorphisms (InDels). They used 50 insertion/ deletion markers (InDels) for distinguishing between *indica* and *japonica* variety. These are co- dominant markers that give two possible alleles according to presence or absence of insertion sequence, situated between the primers.

The present research is envisaged with an objective to access genetic diversity among 19 colored rice genotypes (selected according to the availability of genotypes) linked SSR markers as these markers are highly polymorphic and easy to detect. The INDELs selected were identified from 12 chromosomal locations.

Table 1. List of genotypes used in the present study.

Sr. No	Genotype	Pericarp color
1	RRT IRST 1	RED
2	RRT IRST 2	RED
3	RRT IRST4	RED
4	RRT IRST7	RED
5	RRT IRST 10	RED
6	RRT IRST 13	RED
7	RRT IRST 14	RED
8	RRT IRST 16	RED
9	RRT IRST 19	RED
10	RRT IRST 41	RED
11	RRT IRST 44	RED
12	RRT IRST 47	RED
13	RRT IRST 50	RED
14	TSP	RED
15	A004	BLACK
16	077	BLACK
17	BC1	BROWN
18	*LAL KADA*	RED
19	*KRISHNA KAMOD*	WHITE

Materials and Methods

The study was conducted at Plant Biotechnology Laboratory, Department of Agricultural Botany, B.A College of Agriculture, Anand Agricultural University, Anand during 2011. The seeds of 19 rice genotypes comprising of coloured rice (red, black, brown and white) used in the present study was obtained from the Main Rice Research Station (MRRS), Anand Agricultural University, Nawagam (Table 1). Few healthy seeds were sown and allowed to grow for three weeks in the pots. For the proper growth and emergence of healthy seedlings, these pots were watered regularly and proper light and temperature conditions were maintained. Total genomic DNA extraction from leaves of three weeks old seedlings were carried out by Cetyl trimethyl ammonium bromide (CTAB) method (Ahmadikhah et al., 2006). 300mg tissue samples were homogenized in liquid nitrogen prior mixing with 800 µl of extraction buffer and were incubated for 1 hour at 65º C in water bath. Later on Chloroform: isoamyl alcohol (24:1) was added in the tubes and centrifuged at 4ºC for 15 minutes at 12,000rpm. The supernatant was collected and washed 1-2 times with Chloroform: isoamyl alcohol (24:1) and kept for precipitation with absolute alcohol for overnight. The samples were centrifuged at 5,000 rpm for 15 min to get DNA in pellet form. The pellets were further washed with 70% alcohol and kept for drying and the quality was confirmed through Nanodrop N.D. 1000 software (ver 3.7.1). For SSR and INDEL analysis DNA amplification was performed in 25µl reaction volume containing 50ng genomic DNA, 10X polymerase buffer, 25mM dNTPs, 0.5 µl of each primer (10pmol), 1 unit of Taq DNA polymerase using Applied Biosystem Thermal Cyclers. The cycling conditions were: 1 cycle of 94°C for 7min followed by 40 cycles of 45 sec each of 94°C, 48°C, 72°C and finally 1 cycle of 72°C for 5min. Total of 10 µl aliquots of the amplification products loaded in 3% (w/v) agarose gel for electrophoresis in 1X TBE buffer and stained with Ethidium bromide and documented using SYNGENE GENESNAP G-BOX gel documentation system. These photographs were used to score the DNA bands for analysis. The gels were scored for computer analysis on the basis of the presence and absence of the amplified products. If a product was present in a genotype, it was designated as '1' and if absent, it was designated as '0'. A total of 14 SSR and 21 INDEL markers used for characterizing nineteen rice genotypes. Both SSR and INDEL data were analyzed using NTSYS-PC (Numerical Taxonomical and Multivariant Analysis System computer package). The data were used to generate Jaccard's similarity coefficients for SSR and

INDEL bands. The Jaccard's coefficients between each pair of accessions were used to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA). The PIC, hetrozygosity, and allelic diversity measures marker informativeness and allelic and genotypic frequency were calculated. These measures are calculated using PROC ALLELE procedure of SAS/GENETICS 9.3 version.

Results and Discussion

The present investigation on assessment of pigmented colored rice varieties was carried out in the Biotechnology Laboratory of Department of Agricultural Botany, B. A. College of Agriculture, Anand Agriculture University, Anand, Gujarat, India. Total of 14 SSR and 21 INDEL markers were used for the study out of which 9 SSR markers and 12 INDEL markers gave polymorphic results (Table 2).

Microsatellite (SSR) markers are the PCR based markers that have been developed in many plant species; they have an advantage of being multi allelic, highly polymorphic and codominant. In the present study total of 14 primers were used to generate fingerprint of 19 genotypes of *Oryza sativa* among which nine succeeded to produce polymorphic or monomorphic alleles when applied with rice cultivars generating 129 bands. The level of polymorphism among the varieties was evaluated by calculating the PIC values for each of the nine microsatellite markers. Among the nine microsatellite markers used lowest PIC of 0.36 was recorded in RM 484 while highest PIC of 0.78 was obtained with RM 167. The Cluster analysis divided the genotypes into two major groups A and B. In this group TSP and IRST50 showed highest genetic similarity. First cluster (A) was further sub-divided into two minor clusters i.e., A1 and A2. Second major cluster B included two minor clusters B1 and B2 (Figure 1). The second major cluster indicated that black and brown rice are genetically more closely related to red rice. Highest genetic diversity was shown between *Krishna Kamod* and IRST1 whereas least genetic diversity was observed between *Lal Kada* and *Krishna Kamod,* IRST1 and IRST2. In the present study multiple loci were detected by RM 166, RM484, RM154, RM174, RM167 and RM102. Occurrence of rare alleles might have resulted from unequal crossing over, translocation, other types of mutation or residual heterozygosity (Lapitan et al., 2007). Similar work has been carried out by Rajguru et al. (2005) where genetic diversity between white and red rice varieties was studied through SSR markers. They also found substantial difference between colored rice with microsatellite markers.

Table 2. list of SSR and INDEL primers for characterizing 19 genotypes.

SSR primers	Sequence	INDEL primers	Sequence
RM 166	GGTCCTGGGTCAATAATTGGGTTACC TTGCTGCATGATCCTAAACCGG	R3M37	GCATTGAATTGTACTCTTATTATAT ACGAATCAAAAGGAGACTAAAAT
RM 206	CCCATGCGTTTAACTATTCT CGTTCCATCGATCCGTATGG	R5M13	GAGAAAGAGTGGAAGGAG AGTATCGTCAGGAGGGTC
RM 231	CCAGATTATTTCCTGAGGTC CACTTGCATAGTTCTGCATTG	R6M30	CACAAGCCGTAGCAGAGC TCACGAAAAAGACCCCAAG
RM 26	GAGTCGACGAGCGGCAGA CTGCGAGCGACGGTAACA	R7M20	GTTTTGTGCATTCCTTTAC TTTATGACATTTTGACCG
RM 484	TCTCCCTCCTCACCATTGTC TGCTGCCCTCTCTCTCTCTC	R8M33	CGAAAGAGGAGAGGGGTAGT CGAAAACGAGAAACAAATA
RM154	ACCCTCTCCGCCTCGGCCTCCTC CTCCTCCTCCTGCGACCGCTCC	R8M46	CAGCAGAGTCCAGAGAAGAT GCATAAGATGGCGAGTGA
RN 174	AGCGACGCCAAGACAAGTCGGG TCCACGTCGATCGACACGACGG	R9M10	CTTTGGATTCAGGGGGA AACTTGAAACGGAGGCAG
RM 167	GGTCCTGGGTCAATAATTGGGTTACC TTGCTGCATGATCCTAAACCGG	R9M20	ACTGCTTTGATGGCTTGTG CTCCCCAAACTGAATCC
RM 102	AACTTTCCCACCACCACCGCGG AGCAGCAGCAAGCCAGCAAGCG	R10M10	GAATACAACCCCCTAAAAAC ATGGACCGTTGAGGAGAC
		R10M30	CCCTAAAAATAGAGCAACCT ACCCATAATACTACCAATCAAC
		R11M23	AAGGTTGACAAGGACAGAAG TCGCAGGAATGGATAAAA
		R12M43	CCGCCGAGAAGAAACAAAG CCGCCGAGAAGAAACAAAG

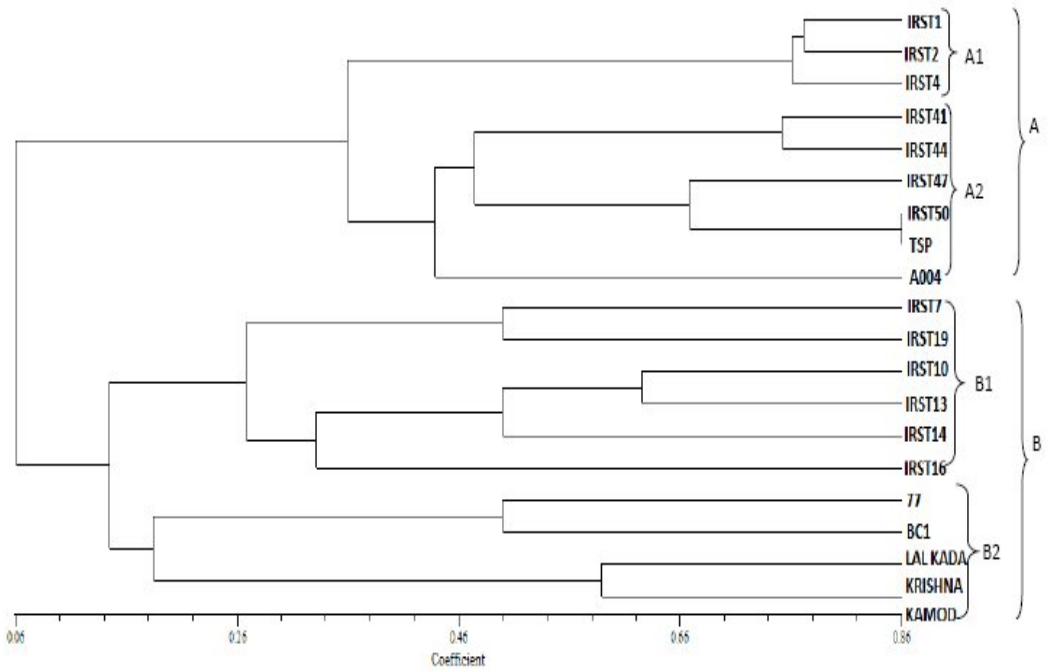

Figure 1. Dendrogram of genetic relationship among rice genotypes based on SSR markers.

A total of 21 INDEL markers were screened out of which only 12 INDEL markers showed expected results. Highest numbers of polymorphic bands were observed in R5M13, R8M46 and R9M10. The highest PIC of 0.81 was recorded in R9M10 while lowest of 0.50 was obtained in R9M20. The level of polymorphism among the varieties was evaluated by calculating the number and PIC values for each of the 9 microsatellite markers evaluated. The dendrogram divides the varieties into two major groups i.e. A and B. Line between the genotypes IRST2 and IRST4 indicates that they have highest genetic similarity. First cluster (A) was further sub-divided into two minor clusters, first minor cluster (A1) comprised of IRST1, IRST2, IRST4, IRST7 and IRST10. Second A2 comprised of IRST13 and IRST 14. Second major cluster (B) included three minor clusters, first minor cluster (B1) comprised IRST16, IRST19, IRST41 and IRST44. Second minor cluster (B2) included IRST47, IRST50, TSP, and A004. While third minor cluster (B3) consisted of 077, BC1, *Lal Kada*, *Krishna Kamod*. Second major cluster depicted black and brown rice, which were genetically closely related to red rice (Figure 2). Similar work has been carried out by Yu et al. (2002) where SSR and INDEL markers were used for diversity between several rice genotypes. Xingxing et al. (2007) validated the INDEL primer pairs based on the comparative genomic study on DNA sequences between several rice species. The result revealed that out of 45 INDEL primers used 41 could accurately identify *indica* and *japonica* rice varieties with reliability over 80%.

All genotypes clustered at a similarity index of 0.05 for SSR and 0.03 for INDEL. The similarity index values for SSR ranged from 0.56 to 0.95 (Table 3). Highest similarity (0.95) observed between IRST41 and IRST44, IRST50 and TSP whereas lowest similarity (0.56) obtained between IRST47 and LAL KADA, IRST4 and LAL KADA. Similarity index for INDEL ranged from 0.00 to

0.88 (Table 4). Highest similarity (0.88) found between IRST2 and IRST4.Average similarity index observed was 0.74 in SSR whereas 0.17 in INDEL. Thus, it can be inferred that less diversity was detected using SSR markers as it is evident from its similarity value.

Arithmetic mean heterozygosity Allelic diversity, polymorphism information content (PIC) and marker indices (MI) are measures for detecting polymorphism and comparing efficiency of markers. PIC is a quantification of the number of alleles that a marker has and the frequency of each of the alleles in the subset of germplasm tested. Since, a marker with fewer alleles has less power to distinguish several samples, and alleles present at low frequency have less power to distinguish, a higher PIC is assigned to a marker with many alleles and with alleles present at roughly equal proportions in the population (Jiang et al., 2010). Among 9 SSR markers and 12 INDEL markers highest PIC value was obtained for INDEL marker R9M10 0.81.

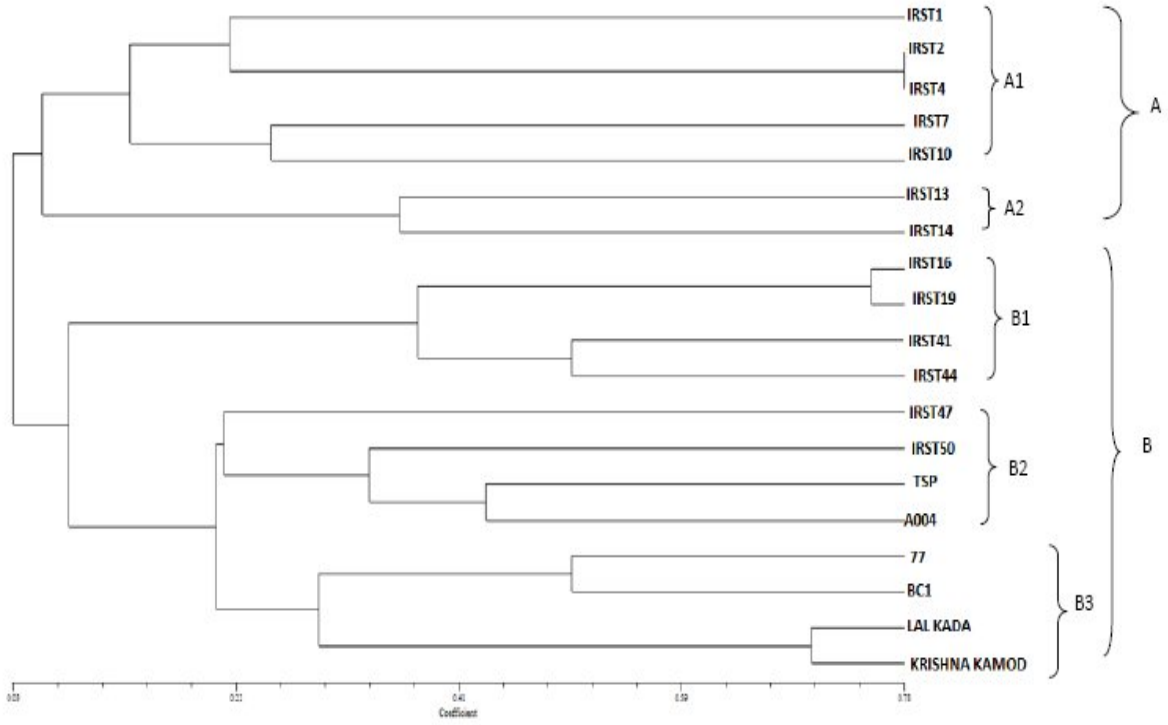

Figure 2. Dendrogram of genetic relationship among rice genotypes based on INDEL markers.

Emir. J. Food Agric. 2014. 26 (6): 497-507
doi: 10.9755/ejfa.v26i6.15722
http://www.ejfa.info/

Table 3. Similarity matrix of rice genotypes based on SSR markers.

	IRST1	IRST2	IRST4	IRST7	IRST10	IRST13	IRST14	IRST16	IRST19	IRST41	IRST44	IRST47	IRST50	TSP	A004	77	BC1	*Lal Kada*	*Krishna Kamod*
IRST1	1																		
IRST2	0.92	1.00																	
IRST4	0.90	0.92	1.00																
IRST7	0.69	0.77	0.69	1.00															
IRST10	0.59	0.67	0.59	0.79	1.00														
IRST13	0.59	0.67	0.59	0.79	0.85	1.00													
IRST14	0.64	0.67	0.59	0.74	0.74	0.85	1.00												
IRST16	0.74	0.82	0.74	0.90	0.79	0.85	0.85	1.00											
IRST19	0.69	0.77	0.69	0.90	0.74	0.79	0.74	0.90	1.00										
IRST41	0.74	0.82	0.74	0.85	0.69	0.74	0.69	0.85	0.90	1.00									
IRST44	0.74	0.82	0.74	0.85	0.69	0.74	0.69	0.85	0.90	0.95	1.00								
IRST47	0.79	0.87	0.79	0.69	0.64	0.64	0.64	0.74	0.74	0.79	0.79	1.00							
IRST50	0.77	0.85	0.77	0.77	0.67	0.72	0.67	0.77	0.82	0.87	0.87	0.87	1.00						
TSP	0.77	0.85	0.77	0.77	0.72	0.77	0.67	0.77	0.82	0.87	0.87	0.87	0.95	1.00					
A004	0.82	0.85	0.77	0.82	0.67	0.72	0.72	0.82	0.82	0.87	0.87	0.77	0.85	0.85	1.00				
77	0.67	0.74	0.67	0.82	0.77	0.77	0.77	0.87	0.82	0.77	0.77	0.67	0.69	0.69	0.74	1.00			
BC1	0.72	0.79	0.72	0.82	0.77	0.77	0.77	0.87	0.82	0.82	0.82	0.72	0.74	0.74	0.79	0.90	1.00		
Lal Kada	0.56	0.64	0.56	0.67	0.62	0.67	0.72	0.72	0.67	0.67	0.67	0.56	0.64	0.59	0.64	0.79	0.74	1.00	
Krishna Kamod	0.59	0.67	0.59	0.74	0.69	0.59	0.69	0.74	0.69	0.69	0.69	0.59	0.62	0.62	0.67	0.72	0.72	0.82	1.00

Table 4. Similarity matrix of rice genotypes based on INDEL markers.

	IRST1	IRST2	IRST4	IRST7	IRST10	IRST13	IRST14	IRST16	IRST19	IRST41	IRST44	IRST47	IRST50	TSP	A004	77	BC1	*Lal Kada*	*Krishna Kamod*
IRST1	1.00																		
IRST2	0.38	1.00																	
IRST4	0.33	0.88	1.00																
IRST7	0.14	0.33	0.43	1.00															
IRST10	0.14	0.17	0.14	0.40	1.00														
IRST13	0.33	0.13	0.11	0.00	0.14	1.00													
IRST14	0.21	0.00	0.00	0.00	0.13	0.53	1.00												
IRST16	0.00	0.14	0.13	0.17	0.17	0.25	0.24	1.00											
IRST19	0.00	0.14	0.13	0.17	0.17	0.13	0.24	0.86	1.00										
IRST41	0.00	0.15	0.13	0.18	0.18	0.00	0.00	0.62	0.62	1.00									
IRST44	0.13	0.31	0.27	0.18	0.18	0.13	0.00	0.46	0.46	0.67	1.00								
IRST47	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.14	0.14	0.31	0.31	1.00							
IRST50	0.11	0.13	0.11	0.00	0.00	0.11	0.00	0.13	0.13	0.27	0.40	0.38	1.00						
TSP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.17	0.46	0.53	1.00					
A004	0.15	0.18	0.15	0.00	0.00	0.15	0.00	0.00	0.00	0.20	0.40	0.18	0.46	0.60	1.00				
77	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.15	0.15	0.17	0.17	0.46	0.27	0.50	0.40	1.00			
BC1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.15	0.17	0.17	0.15	0.27	0.33	0.40	0.67	1.00		
Lal Kada	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.13	0.13	0.38	0.22	0.40	0.31	0.53	0.40	1.00	
Krishna Kamod	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.14	0.27	0.24	0.43	0.33	0.43	0.43	0.82	1.00

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A more specific comparison of overall efficiency of two marker systems was provided by marker index (MI). Comparison of the marker index values revealed high level of polymorphism in INDEL (0.23) than SSR (0.19) (Tables 5, 6). Mean allelic diversity for 9 SSR markers were found to be 0.67 and 0.64 for 12 INDEL markers which indicated that the diversity detected by INDEL was more than SSR. The arithmetic mean heterozygosity across all loci was 0.61 and 0.60 for SSR and INDEL marker respectively. All the measures used to compare the efficiency of the two markers indicated that there was higher level of polymorphism detected by INDEL than SSR markers, thus it can be concluded that INDEL markers was more efficient than SSR in the present study.

Table 5. SSR marker analysis by statistical analysis system (SAS) software.

Marker Summary									Genotype Frequencies		
Locus	Number of Individuals	Number of Alleles	Allele	Count	Frequency	Polymorph Info Content	Heterozygosity	Allelic Diversity	Genotype	Count	Frequency
RM 166	7	5	A	4	0.2857	0.7397	0.0000	0.7755	A/A	2	0.2857
			B	4	0.2857				B/B	2	0.2857
			C	2	0.1429				C/C	1	0.1429
			D	2	0.1429				D/D	1	0.1429
			E	2	0.1429				E/E	1	0.1429
RM 206	10	6	A	6	0.3000	0.7716	0.0000	0.8000	A/A	3	0.3000
			B	2	0.1000				B/B	1	0.1000
			C	4	0.2000				C/C	2	0.2000
			D	4	0.2000				D/D	2	0.2000
			E	2	0.1000				E/E	1	0.1000
			F	2	0.1000				F/F	1	0.1000
RM 231	18	5	A	6	0.1667	0.6681	0.0000	0.7099	A/A	3	0.1667
			B	16	0.4444				B/B	8	0.4444
			C	8	0.2222				C/C	4	0.2222
			D	2	0.0556				D/D	1	0.0556
			E	4	0.1111				E/E	2	0.1111
RM 26	13	3	B	16	0.6154	0.4836	0.0000	0.5444	B/B	8	0.6154
			C	4	0.1538				C/C	2	0.1538
			D	6	0.2308				D/D	3	0.2308
RM 484	14	2	A	16	0.5714	0.3698	0.0000	0.4898	A/A	8	0.5714
			B	12	0.4286				B/B	6	0.4286
RM 154	11	3	A	6	0.2727	0.5262	0.0000	0.5950	A/A	3	0.2727
			B	4	0.1818				B/B	2	0.1818
			C	12	0.5455				C/C	6	0.5455
RM 174	17	4	A	10	0.2941	0.6457	0.0000	0.6990	A/A	5	0.2941
			B	4	0.1176				B/B	2	0.1176
			C	14	0.4118				C/C	7	0.4118
			D	6	0.1765				D/D	3	0.1765
RM 167	19	7	A	4	0.1053	0.7843	0.0000	0.8089	A/A	2	0.1053
			B	6	0.1579				B/B	3	0.1579
			C	6	0.1579				C/C	3	0.1579
			D	6	0.1579				D/D	3	0.1579
			E	2	0.0526				E/E	1	0.0526
			F	12	0.3158				F/F	6	0.3158
			G	2	0.0526				G/G	1	0.0526
RM 102	6	3	A	2	0.1667	0.5355	0.0000	0.6111	A/A	1	0.1667
			B	6	0.5000				B/B	3	0.5000
			C	4	0.3333				C/C	2	0.3333

Table 6. INDEL marker analysis by statistical analysis system (SAS) software.

Marker Summary									Genotype Frequencies		
Locus	Genotype	Count	Frequency	Count	Frequency	Polymorph Info Content	Heterozygosity	Allelic Diversity	Genotype	Count	Frequency
R3M37	1	1	A	2	1.0000	0.0000	0.0000	0.0000	A/A	1	1.0000
			A	2	0.1429				A/A	1	0.1429
R5M13	7	4	B	4	0.2857	0.6414	0.0000	0.6939	B/B	2	0.2857
			C	2	0.1429				C/C	1	0.1429
			D	6	0.4286				D/D	3	0.4286
			A	4	0.2500				A/A	2	0.2500
R6M30	8	3	B	4	0.2500	0.5547	0.0000	0.6250	B/B	2	0.2500
			C	8	0.5000				C/C	4	0.5000
			A	20	0.5882				A/A	10	0.5882
R7M20	17	4	B	2	0.0588	0.5290	0.0000	0.5813	B/B	1	0.0588
			C	8	0.2353				C/C	4	0.2353
			D	4	0.1176				D/D	2	0.1176
			A	2	0.2500				A/A	1	0.2500
R8M33	4	4	B	2	0.2500	0.7031	0.0000	0.7500	B/B	1	0.2500
			C	2	0.2500				C/C	1	0.2500
			D	2	0.2500				D/D	1	0.2500
			A	14	0.3684				A/A	7	0.3684
			B	10	0.2632				B/B	5	0.2632
R8M46	19	5	C	4	0.1053	0.7085	0.0000	0.7479	C/C	2	0.1053
			D	4	0.1053				D/D	2	0.1053
			E	6	0.1579				E/E	3	0.1579
			A	4	0.2857				A/A	2	0.2857
R9M10	7	3	B	2	0.1429	0.5015	0.0000	0.5714	B/B	1	0.1429
			C	8	0.5714				C/C	4	0.5714
R9M20	14		A	2	0.0714				A/A	1	0.0714
		4	B	10	0.3571	0.5407	0.0000	0.6122	B/B	5	0.3571
			C	14	0.5000				C/C	7	0.5000
			D	2	0.0714				D/D	1	0.0714
			A	2	0.1000				A/A	1	0.1000
			B	2	0.1000				B/B	1	0.1000
			C	2	0.1000				C/C	1	0.1000
R10M10	10	7	D	6	0.3000	0.7978	0.0000	0.8200	D/D	3	0.3000
			E	2	0.1000				E/E	1	0.1000
			F	2	0.1000				F/F	1	0.1000
			G	4	0.2000				G/G	2	0.2000
			A	2	0.0714				A/A	1	0.0714
			C	2	0.0714				C/C	1	0.0714
			D	2	0.0714				D/D	1	0.0714
R10M30	14	8	E	8	0.2857	0.8058	0.0000	0.8265	E/E	4	0.2857
			F	6	0.2143				F/F	3	0.2143
			G	2	0.0714				G/G	1	0.0714
			H	2	0.0714				H/H	1	0.0714
			I	4	0.1429				I/I	2	0.1429
		5	A	10	0.4167				A/A	5	0.4167
			B	4	0.1667				B/B	2	0.1667
R11M23	12		C	4	0.1667	0.6990	0.0000	0.7361	C/C	2	0.1667
			D	4	0.1667				D/D	2	0.1667
			E	2	0.0833				E/E	1	0.0833
			A	6	0.1667				A/A	3	0.1667
			B	16	0.4444				B/B	8	0.4444
R12M43	18	5	C	4	0.1111	0.6859	0.0000	0.7222	C/C	2	0.1111
			D	4	0.1111				D/D	2	0.1111
			E	6	0.1667				E/E	3	0.1667

Conclusion

Characterization and quantification of genetic diversity have been a major goal in evolutionary biology. Information of genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. Information regarding genetic variability at molecular level could be used to help, indentify and develop genetically unique germplasm that compliments existing cultivars. The present work will be a boon for plant breeders in choosing the varieties for generating a new hybrid. It can be concluded that among 19 genotypes, the preferable genotype for crossing is IRST 1 and *Krishna kamod* as it has highest genetic diversity and thus maximum heterosis can be exploited by crossing the varieties IRST 1 and *Krishna kamod*.

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