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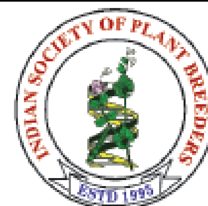

Research Article

Molecular diversity analysis in rice (*Oryza sativa* L.) using SSR markers

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Abstract
Rice is the most important staple food crop among the cereals and feeds more than half of the world's population. Assessment of genetic diversity is of utmost importance in rice breeding from the perspective of selection, conservation and proper utilization. The present study was undertaken with an objective to assess the genetic diversity among 27 rice cultivars with 12 SSR markers. The results revealed a total of 40 alleles were detected across 27 rice cultivars tested. PIC values varied widely among SSR loci tested and it ranged from 0.38 to 0.65, with an average of 0.56 per marker. The 27 rice cultivars were grouped into two major clusters *i.e.,* cluster I and II with similarity coefficient 0.13. Cluster I was sub divided into two minor sub-groups IA and IB having 5 and 8 genotypes respectively. These subgroups were further subdivided into minor groups. In similar way, the second main cluster *i.e.* Cluster II was also sub divided into two minor sub-groups that is IIA and IIB having 5 and 9 genotypes respectively. These subgroups were further divided into minor groups. This indicated presence of considerable diversity in the genotypes studied and the most diverse cultivars were IR 98846-2-1-2-3 and IR 14D201.

Key words
Rice, dendrogram, molecular diversity, SSR markers

INTRODUCTION

Rice is the grain of life for more than 70 per cent of the Asian population and it has great prominence in Indian culture. Worldwide, rice is grown on an area of 163 million hectares with a production of 751.9 million tonnes and with an average productivity of 4.52 tonnes per hectare (FAO Rice Market Monitor, 2017), out of which 90 per cent of world's rice is produced and consumed in Asia. To feed the ever-growing population, the targeted rice production of the world, China and India for the year 2030 is envisioned as 771.02, 168.90 and 130.02 million tonnes respectively (USDA, 2014). To get success in accomplishing the target, the increase in rice productivity is the only option left, since the other alternatives like cultivable land, water and other natural resources are either stagnant or declining (Singh *et al*. 2013). The average yield of hybrid rice is at least 15-20% more than that of inbred rice and it has been expected that hybrid rice technology will play a crucial role in ensuring food security worldwide in the forthcoming decades (Sabar and Akhter 2007). Thus, there is a crucial need to boost the rice production through its enhanced productivity which may be done by the breeders through adaptation of hybrid rice technology at larger scale. A key factor for the crop improvement effort depends upon the amount and the use of genetic variability in breeding programs.

Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for evaluating changes in genetic diversity over time and space (Duvick 1984). Molecular markers are very powerful tools for evaluation of genetic diversity. Numerous molecular markers are being used for fingerprinting and diversity analysis

among crop plants such as Restriction Fragment Length Polymorphism (RFLP) (Sun *et al*. 2001), Random Amplified Polymorphic DNA (RAPD) (Williams *et al.* 1990), microsatellites or Simple Sequence Repeats (SSRs) (Gao *et al*. 2005) and Amplified Fragment Length Polymorphism (AFLP) (Aggarwal *et al*. 1999). Simple sequence Repeat (SSR) is a vital tool for genetic variation identification of germplasm (Powell *et al*. 1996) and hence, being extensively applied in germplasm diversity analysis (Jin *et al.* 2010), identification of heterosis, genetic relationship between species etc. A total of 18,828 Class 1- di, tri, and tetra-nucleotide SSRs, representing 47 distinct motif families, have been identified and annotated on the rice genome (International Rice Genome Sequencing Project, 2005). Hybrid rice technology is one of the sturdiest tools to break the yield barriers. As diversity is one of the major criteria to get the higher magnitude of heterosis in the F_1 hybrids, it is necessary to study the genetic diversity among the rice genotypes to make the heterosis breeding programme practically useful. In the light of above points, the present investigation was undertaken to study genetic diversity among 27 rice cultivars so as to identify varying genotypes which would aid in the rice breeding programmes.

MATERIALS AND METHODS

The experimental material for this investigation comprised of 27 rice genotypes (**Table 1**). DNA was extracted from 20-25 days old leaves of each variety, following CTAB extraction method according to Doyle and Doyle, 1987 with few modifications. Twelve SSR markers were used for genetic diversity analysis (**Table 2**). The markers were selected based on earlier screening (work not published). PCR amplification was carried out following the standard procedures. The amplified products were visualized and photographed under UV light source in a gel documentation system.

Table 1. List of rice genotypes used in present study

S. No.	Genotypes	Source of Seeds	S. No.	Genotypes	Source of Seeds
1.	IR98846-2-1-2-3	IRRI, South Asia Hub	14.	IR 87959-6-2-3-1-2-BAY	IRRI, South Asia Hub
2.	IR 93349:13-B-8-5-4-1RGA-2RGA-1-B-B	IRRI, South Asia Hub	15.	IRRI 123	IRRI, South Asia Hub
3.	CRR 751-1-10-B-B	IRRI, South Asia Hub	16.	SAHABAGIDHAN	IRRI, South Asia Hub
4.	IR 90257-B-577-B-B	IRRI, South Asia Hub	17.	IR 64	IRRI, South Asia Hub
5.	Local check (NDR 359)	NDUAT, Faizabad	18.	IR 93328:25-B-19-12-15-1RGA-2RGA-1-B	IRRI, South Asia Hub
6.	IR 90257-B-272-B-B-B	IRRI, South Asia Hub	19.	IR 14D 201	IRRI, South Asia Hub
7.	TRP-27-10-1-B-B	IRRI, South Asia Hub	20.	IR 93354:20-B-17—8-1RGA-2RGA-1-B-B	IRRI, South Asia Hub
8.	IR 98816-10-2-2-3	IRRI, South Asia Hub	21.	IR 93356:6-B-11-6-22-1RGA-2RGA-1-B-B	IRRI, South Asia Hub
9.	IR 90257-B-577-1-B-B	IRRI, South Asia Hub	22.	IR 15L 1061	IRRI, South Asia Hub
10.	IR 97076-30-1-1-2	IRRI, South Asia Hub	23.	BPT-5204	APAU, Hyderabad
11.	IR 93328:15-B-8-12-14-1RGA-2RGA-1-B-B	IRRI, South Asia Hub	24.	AKSHAYDHAN	IIRR, Hyderabad
12.	IR 107855-13:24-B-B	IRRI, South Asia Hub	25.	DANTESHWARI	IGKV, Raipur
13.	IR 97044-6-2-1-2	IRRI, South Asia Hub	26.	HUR-3022	BHU, Varanasi
			27.	PUSA-6B	IARI, New Delhi

Band position in comparative SSR profile for each genotype and primer combination was scored from the respective gel images. SSR profile of those genotype × primer combination, which gave constant amplification for all the genotype and without any blank lane per unclear bands, was only included in this study. The amplified fragments were scored as '1' for the presence and '0' for the absence of a band generating the 0 and 1 matrix. These binary data matrix was then utilized to generate genetic similarity data among the 27 lines of rice genotypes. The binary data matrix generated by polymorphic SSR markers were subjected to further analysis using NTSYS-pc version 2.11W (Rohlf 1997). The SIMQUAL programme was used to calculate the Jaccard dissimilarity coefficient. The dissimilarity matrix was used as an input for analysis of clusters. UPGMA-based clustering was done using Sequential Agglomerative Hierarchial Non-Overlapping (SAHN) module and utilizing Jaccard's Co-efficient of NTSYSpc for dendrogram construction. In Un-weighted pair-group method with arithmetic averages (UPGMA), clusters were joined based on the average distance between all members in the two groups.

PIC value of markers was calculated. The calculation of PIC (Weir 1996) for the *i*th marker is PIC = $1 - \Sigma\, Pij$ ($j=1, 2,...n$), where *Pij* is the frequency of the jth pattern

Table 2 List of SSR markers used for genetic diversity analysis

Marker Name	Chromosome No.	Forward/ Reverse	Sequence 5'-------------> 3'	Temp. (⁰C)
RM315	1	Forward	GAGGTACTTCCTCCGTTTCAC	59.8
		Reverse	AGTCAGCTCACTGTGCAGTG	59.4
RM443	1	Forward	GATGGTTTTCATCGGCTACG	57.3
		Reverse	AGTCCCAGAATGTCGTTTCG	57.3
RM171	10	Forward	AACGCGAGGACACGTACTTAC	59.8
		Reverse	ACGAGATACGTACGCCTTTG	57.3
RM6100	10	Forward	TCCTCTACCAGTACCGCACC	61.4
		Reverse	GCTGGATCACAGATCATTGC	57.3
RM312	1	Forward	GTATGCATATTTGATAAG	49.1
		Reverse	AAGTCACCGAGTTTACCT	55.3
RM5	1	Forward	TGCAACTTCTAGCTGCTC	57.3
		Reverse	GCATCCGATCTTGATGG	56
RM144	11	Forward	TGCCCTGGCGCAAATTTGATCC	62.1
		Reverse	GCTAGAGGAGATCAGATGGTAGTG	62.7
RM19	12	Forward	CAAAAACAGAGCAGATGAC	52.4
		Reverse	CTCAAGATGGACGCCAAGA	56.7
RM55	3	Forward	CCGTCGCCGTAGTAGAGAAG	61.4
		Reverse	TCCCGGTTATTTTAAGGCG	54.5
RM510	6	Forward	AACCGGATTAGTTTCTCGCC	57.3
		Reverse	TGAGGACGACGAGCAGATTC	59.4
RM11943	1	Forward	CTTGTTCGAGGACGAAGATAGGG	57
		Reverse	CCAGTTTACCAGGGTCGAAACC	57
RM431	1	Forward	GCTTGCTTGTATCTGCATTGGTAGG	58
		Reverse	GGGATGATCCACTCTCTGTTTGG	57

for the ith marker and the summation extends over (n) patterns (Peng and Lapitan 2005). The PIC value is an evidence of diversity and frequency among the varieties (Pervaiz *et al.* 2009).

RESULTS AND DISCUSSION

The assessment of genetic diversity is an indispensable component in germplasm characterization and conservation. The results derived from analyses of genetic diversity at the DNA level could be used for planning effective breeding programs aimed at broadening the genetic basis of commercially grown varieties. (Wang and Chee 2010). In the present study, a total of 12 SSR random markers were used to assess the extent of genetic diversity across 27 rice cultivars. Of 12 SSR markers, 11 were found to be polymorphic whereas, one marker, RM312 didn't show amplification. Gel pictures showing polymorphism were shown in Fig. 1 to 3 for some of the markers.

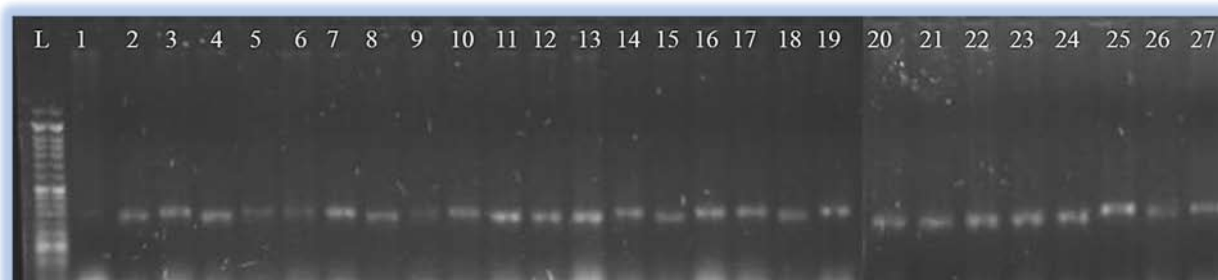

Fig. 1. Banding pattern obtained with SSR marker RM 171

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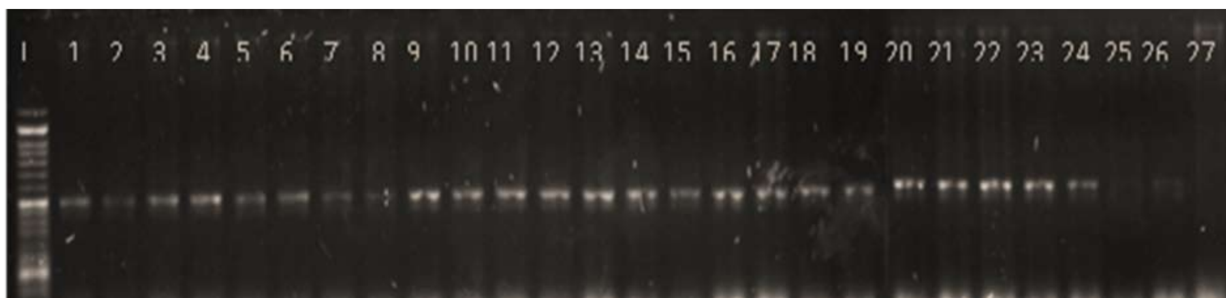

Fig. 2. SSR banding profile obtained by marker RM 431

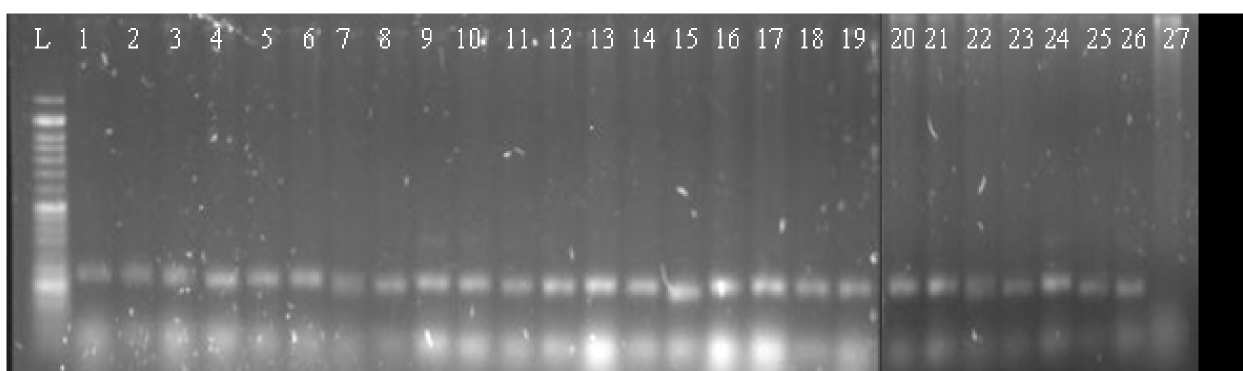

Fig. 3. SSR banding profile obtained by marker RM 19
L = 100 bp Ladder, 1 to 27 are the genotypes used in the present study listed in Table1

A total of 40 alleles were detected across 27 rice cultivars used. The number of alleles generated per locus by each marker ranged from 3-5 with an average of 3.7. The results obtained in the present study were comparable to the observations made by Rabey *et al*. 2013 and Shah *et al.* 2013, where they noticed mean number of alleles were 3.83 and 2.75 in genetic diversity of eight rice varieties and variation within the aromatic and non-aromatic rice cultivars respectively. However, the result obtained from this study was showing more diversity which is higher than the output got by Kibria *et al.* (2009) where with an average number of alleles 1.78 per locus p in order to assess the genetic diversity among aromatic rice genotypes using simple sequence repeat (SSR) and randomly amplified polymorphic DNA (RAPD) markers through marker aided selection (MAS). This obtained result was higher than the result mentioned by Prabakaran *et al.* 2010 had reported the magnitude of average alleles 2.2 per locus in study of genetic polymorphism had done for rice land races. Similarly, the present result was higher than the results reported by (Pachauri *et al*. 2013) with the mean number of alleles per locus detected as 2.79 in molecular and morphological characterization of Indian farmers rice varieties.

A dendrogram (Fig. 4) based on Jaccard's similarity co-efficient was constructed. Cluster Analysis was performed by using UPGMA based on similarity co-efficient values. It resolved 27 rice cultivars lines into two major clusters *i.e.* cluster I and II with similarity coefficient 0.13. Cluster I can be sub divided into two minor sub-groups IA and IB, with similarity coefficient (0.16) having 5 and 8 genotypes respectively. Cluster IA can be further subdivided into two minor subgroups that is, IA-1 and IA-2 (0.195). Cluster IB can be further sub-divided into 2 minor subgroups, IB-1 and IB-2 (0.45). In similar way, the second main cluster *i.e.* Cluster II can be also sub-divided into two minor sub-groups that is IIA and IIB with similarity coefficient (0.263) having 5 and 9 genotypes respectively. Cluster IIA can be further subdivided into 2 minor subgroups that is, IIA-1 and IIA-2 (0.44). Cluster IIB can be further subdivided into 2 minor subgroups, IIB-1 and IIB-2 (0.31). This analysis indicated the presence of considerable diversity in the germplasms studied. The diverse genotypes are therefore, important in order to select the desirable genotypes for utilizing in breeding programmes. On the basis of the dendrogram, the highest similarity was observed between cultivar IR 93328:15-B-8-12-14-1RGA-2RGA-1-B-B and IR 107855-13:24-B-B followed by IR 93354:20-B-17—8-

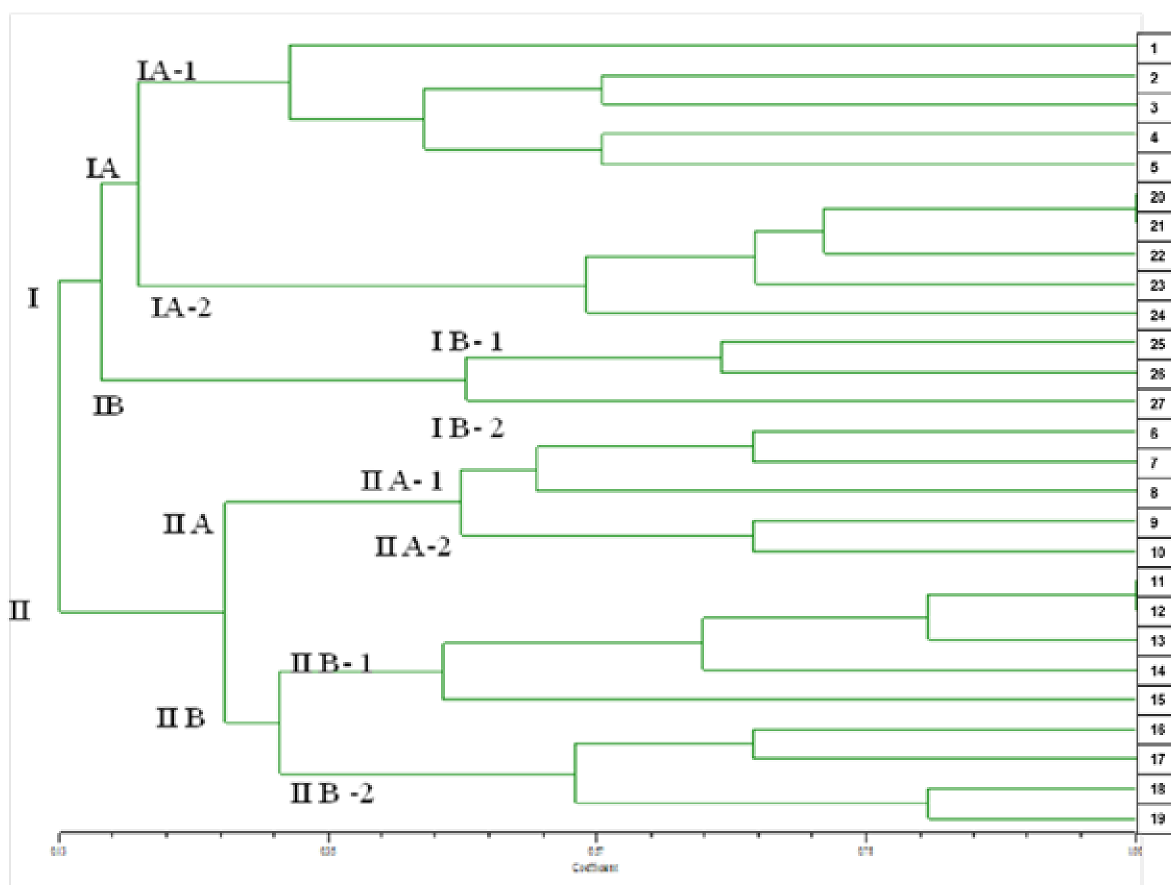

Fig. 4 Cluster analysis with UPGMA method in 27 genotypes of rice using SSR fingerprint data and a Jaccard's similarity matrix.

1 = IR 98846-2-1-2-3, 2 = IR 93349:13-B-8-5-4-1RGA-2RGA-1-B-B, 3 = CRR 751-1-10-B-B, 4 = IR 90257-B-577-B-B, 5 = Local check (NDR 359), 6 = IR 90257-B-272-B-B-B, 7 = TRP-27-10-1-B-B, 8 = IR 98816-10-2-2-3, 9 = IR 90257-B-577-1-B-B, 10 = IR 97076-30-1-1-2, 11 = IR 93328:15-B-8-12-14-1RGA-2RGA-1-B-B, 12 = IR 107855-13:24-B-B, 13 = IR 97044-6-2-1-2, 14 = IR 87959-6-2-3-1-2-BAY, 15 = IRRI 123, 16 = SAHABAGIDHAN, 17 = IR 64, 18= IR 93328:25-B-19-12-15-1RGA-2RGA-1-B-B, 19 = IR 14D 201, 20 = IR 93354:20-B-17—8-1RGA-2RGA-1-B-B, 21 = IR 93356:6-B-11-6-22-1RGA-2RGA-1-B-B, 22 = IR 15L 1061, 23 = BPT-5204, 24 = AKSHAYDHAN, 25 = DANTESHWARI, 26 = HUR-3022, 27 = PUSA-6B

1RGA-2RGA-1-B-B and IR 93356:6-B-11-6-22-1RGA-2RGA-1-B-B . The most diverse cultivars were IR 98846-2-1-2-3 and IR 14D 201. The results were comparable to Upadhyay *et al.* 2011 who had reported clustering of 29 rice genotypes into major clusters while studying the development of molecular tags for rice lines. Similarly, Rajendran *et al*. 2013 studied clustering of maintainer and restorer groups into two different clusters in DNA fingerprinting and estimation of genetic diversity among hybrid rice parental lines (*Oryza sativa* L.). Sonkar *et al.* 2016 also conducted a molecular diversity analysis of 36 rice cultivars using 4 SSR primers and found similar results in which the germplasms were grouped into four clusters. This analysis indicated the presence of considerable diversity in the germplasms studied. The diverse genotypes are therefore, important in order to select desirable genotypes for utilization in breeding programmes.

The similarity coefficient varied from genotypes IR 98846-2-1-2-3 and IR 14D 201. The genotypes, IR 93328:15-B-8-12-14-1RGA-2RGA-1-B-B and IR 107855-13:24-B-B are more similar in origin due to their high similarity coefficient (1.00). Also, the genotypes, IR 93354:20-B-17—8-1RGA-2RGA-1-B-B and IR 93356:6-B-11-6-22-1RGA-2RGA-1-B-B are more similar in origin due to their high similarly coefficient (1.00). Genotype IR 98846-2-1-2-3 is distantly related to Local check (NDR-359) with similarity coefficient (0.222); AKSHAYDHAN with coefficient (0.167) followed by PUSA-6B with coefficient (0.176). Genotype IR 98846-2-1-2-3 showed the highest similarity with IR 93349:13-B-8-5-4-1RGA-2RGA-1-B-B with similarity coefficient (0.467). Genotype IR 93354:20-B-17—8-1RGA-2RGA-1-B-B is distantly related to AKSHAYDHAN with coefficient (0.467) followed by PUSA-6B with coefficient (0.000). Genotype IR 90257-B-272-B-B-B is distantly related to IR 97076-30-1-1-2 with coefficient (0.375) followed by

IR 14D 201 with coefficient (0.222). Genotype IR 90257-B-272-B-B-B showed the highest similarity TRP-27-10-1-B-B with (0.692). Genotype IR 93328:15-B-8-12-14-1RGA-2RGA-1-B-B showed the least similarity with IRRI 123 with (0.467) followed by IR 14D 201 with coefficient (0.222). Similar result was also found by Yadav *et al*. 2013 using 50 QTL linked markers among 88 rice accessions. This result was comparable to those obtained by Sonkar *et al*. 2016 using 4 SSR markers on 36 rice genotypes.

Table 3. Polymorphic information content (PIC) of SSR primers used in the present study

PRIMERS	PIC VALUES
RM 171	0.53
RM 6100	0.65
RM 315	0.59
RM 443	0.63
RM 5	0.60
RM 144	0.54
RM 19	0.62
RM 55	0.38
RM 510	0.57
RM 11943	0.46
RM 431	0.61

In the present study, PIC values varied widely among SSR loci tested and it ranged from 0.38 (RM55) to 0.65 (RM 6100), with an average of 0.56 per marker (**Table 3**). Among the polymorphic SSR markers, RM 6100 showed the highest PIC value 0.65. Markers with PIC values of 0.5 or above are broadly highlights for the genetic studies since they are most useful in describing the polymorphism magnitude of a specific locus (Akkaya and Buyukunal Bal 2004). The obtained results are showing close resemble to the output mentioned by Brondani *et al.* 2008, Rajendrakumar *et al.* 2009 and Matin *et al.* 2012; they also mentioned mean PIC Values of 0.61, 0.61 and 0.634, respectively with variable set of rice genotypes with use of SSR markers. However, the PIC values reported in this study were lower than those obtained by Sonkar *et al.* 2016 who reported a mean PIC value of 0.92. From the above discussion it can be concluded that characterization of germplasm is a prerequisite for crop improvement and systematic study. This study implies that SSR markers are effective tools to discriminate various rice genotypes. Among the cultivars, IR 98846-2-1-2-3 and IR 14D 201 were most diverse.

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