Original Research Article

Molecular characterization of different varieties of rice (*Oryza sativa* L.) using SSR markers

Abstract

The present study was performed to analyze molecular diversity among different varieties of rice using SSR markers, which are effective and reliable tools for this type of analysis. A total of 34 different alleles were generated using 17 SSR primers. Out of these 33 alleles were found to be polymorphic and only one was monomorphic. On average, 2 alleles per primer and 1.94 polymorphic alleles per primer was calculated. In the cluster analysis three varieties KATKIRICE, SONUMRICE and 1010 were found to be diverse from other varieties of rice. These varieties may be used as diverse parents for the future breeding programs for rice improvement. According to Jaccard's similarity coefficient, the highest genetic diversity was observed between KATKIRICE and CHANDRAHASNI. Both varieties were grouped distantly. Out of 17 primers, the best 10 primers were selected based on polymorphic banding patterns for genetic diversity analysis. These selected 10 primers are sufficient to discriminate the group of rice varieties. These findings not only highlight the capacity of the SSR technique but also help in the selection of diverse rice varieties for conservation and crop improvement.

Key words: Agriculture, molecular markers, genetic diversity, similarity, phylogenetic analysis, genotypes

Introduction

Rice (*Oryza sativa* L.) is one of the maximum vital food crops in the world. Around three billion inhabitants consume rice as an essential food which gives on the subject of 50 to 80% of their daily calories. India is considered a big diversity center for rice and reports are available on diversity both at inter and intraspecific levels [1]. In any crop improvement programme, yield, valuable characters and presence of tolerant genes for biotic and abiotic stresses are main targets [2]. These targets have participated in the increase of agricultural production in the country [3]. A common problem with the uninterrupted assortment among genetically related cultivars is the reduction of the hereditary establishment of the crops. The foundation of current agriculture is the choice of genetically diverse genotypes as parents for crossing purposes [4].

Identification of diversity at the genetic level is a pre-requisite for any crossing programme for crop improvement. It contributes to the establishment of genetic affiliation in the collection of germplasm. This identification also helps in the selection of diverse parental combinations with higher genetic variation and possibilities of recombination to transfer advantageous genes [5,6]. At present plentiful molecular markers are available to estimate the genetic variation and create the molecular fingerprint of rice genotypes. Among all markers, simple sequence repeat (SSR) markers have proved their efficiency in rice for the estimation of genetic variation [7]. Several researchers have used SSR markers in the molecular diversity analysis of rice. SSR based fingerprinting allows rapid cultivar classification, which is a proven tool for genotypic categorization, assortment, and execution [8,9]. Additionally, SSR markers have their own benefits in comparison to single nucleotide polymorphism (SNP) markers for genetic diversity analysis at a low cost [10].

The objectives of the present investigation were to analyze the molecular variability and genetic relatedness of 45 rice genotypes using SSR markers. This study will improve our understanding of genetic diversity among different genotypes of rice and make possible the exploitation of dissimilar genotypes in rice improvement.

Materials and Methods

Rice materials

A total of 45 indica inbred rice varieties were chosen to represent a wide diversity (Table-1). These materials were collected from different locations of Madhya Pradesh and Chhattisgarh.

Table-1 Rice varieties used in the present study

Sl.	Varieties	Location	Place	State	Latitude	Longitude
1	SARNA	Local market	Chhuikhdan	Chhattisgarh	21.5251° N	80.9956° E
2	BASMATIPS3	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
3	IGKVR2	IGKVV	Raipur	Chhattisgarh	21.2376° N	81.7055° E
4	1010	Local market	Chhuikhdan	Chhattisgarh	21.5251° N	80.9956° E
5	KARIKAMOD	Local market	Chhuikhdan	Chhattisgarh	21.5251° N	80.9956° E
6	IR64	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
7	IGKVR1	IGKVV	Raipur	Chhattisgarh	21.2376° N	81.7055° E
8	CULTURERICE	Local market	Chhuikhdan	Chhattisgarh	21.5251° N	80.9956° E
9	AMPORICE	Local market	Chhuikhdan	Chhattisgarh	21.5251° N	80.9956° E
10	SAMBLESHWARI	IGKVV	Raipur	Chhattisgarh	21.2376° N	81.7055° E
11	DUBRAJ	Local market	Chhuikhdan	Chhattisgarh	21.5251° N	80.9956° E
12	BASMATIP1460	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
13	MTU1010	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
14	CHANDRAHASNI	IGKVV	Raipur	Chhattisgarh	21.2376° N	81.7055° E
15	DANTESHWARI	IGKVV	Raipur	Chhattisgarh	21.2376° N	81.7055° E
16	MTU1011	Local market	Chhuikhdan	Chhattisgarh	21.5251° N	80.9956° E
17	KAROKAMOL	Local market	Chhuikhdan	Chhattisgarh	21.5251° N	80.9956° E
18	SATARISFARI	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
19	SONAMRICE	Local market	Birutola	Chhattisgarh	21.4973° N	80.9867° E
20	BAIJARRICE	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
21	HATHWA	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
22	JHIGAFULL	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
23	BAMLESHWARI	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
24	LALURICE	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
25	M2	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
26	SUKNANDAN	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
27	WGL32100	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
28	IR81	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
29	KRANTI	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
30	SAFADSARNA	Local market	Birutola	Chhattisgarh	21.4973° N	80.9867° E
31	SAHBAGHI	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
32	JR201	JNKVV	Jabalpur	Madhya Pradesh	23.2072° N	79.9540° E
33	CHINDEMORI	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
34	KATKIRICE	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
35	SURJARICE	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
36	KARHANI	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
37	GHINMORI	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
38	HMT	Local market	Birutola	Chhattisgarh	21.4973° N	80.9867° E
39	RANIKAJAL	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
40	IGKVR1244	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
41	KANASHRI	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
42	GERAFULL	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
43	SATHARHSAFRI	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E
44	MAHAMAYA	Local market	Birutola	Chhattisgarh	21.4973° N	80.9867° E
45	CHOTISAFRI	KVK	Korea	Chhattisgarh	23.2713° N	82.5565° E

Genomic DNA extraction and molecular analysis

Leaf samples were collected after transplanting. DNA was extracted following the CTAB procedure as described by Doyle and Doyle [11] with required modifications. Total 17 SSR primers (Table-2) were used to detect polymorphism of selected genotypes. The primers were procured from Integrated DNA Technology, USA. PCR was performed in a 10µL reaction mixture containing 25 ng template DNA, 0.5 µmol/L of each primer, 200 µmol/L of

each dNTP, 1.5 mmol/L $MgCl_2$ and 1U *Taq* polymerase and 1.0 µL of 10x PCR reaction buffer. The PCR procedure was done as 5 min initial denaturation at 94 °C; 35cycles of 30 sec denaturation at 94 °C, 30 sec anneal at 55 °C and 1 min extension at 72 °C; and 5 min final extension at 72 °C. The amplified products were electrophoresed on 2.5% agarose gel.

Table-2 SSR primers and their sequences used in the study

Sl.	Primer	Forward 5'-3'	GC %	Reverse 5'-3'	GC %
1	RM474	AAGATGTACGGGTGGCATTC	50	TATGAGCTGGTGAGCAATGG	50
2	RM413	GGCGATTCTTGGATGAAGAG	50	TCCCCACCAATCTTGTCTTC	50
3	RM484	TCTCCCTCCTCACCATTGTC	55	TGCTGCCCTCTCTCTCTCTC	60
4	RM19	CAAAAACAGAGCAGATGAC	42.1	CTCAAGATGGACGCCAAGA	52.6
5	RM212	CCACTTTCAGCTACTACCAG	50	CACCCATTTGTCTCTCATTATG	40.9
6	RM249	GGCGTAAAGGTTTTGCATGT	45	ATGATGCCATGAAGGTCAGC	50
7	RM250	GGTTCAAACCAAGCTGATCA	45	GATGAAGGCCTTCCACGCAG	60
8	RM280	ACACGATCCACTTTGCGC	55.5	TGTGTCTTGAGCAGCCAGG	57.8
9	RM541	TATAACCGACCTCAGTGCCC	55	CCTTACTCCCATGCCATGAG	55
10	RM11	TCTCCTCTTCCCCCGATC	61.1	ATAGCGGGCGAGGCTTAG	61.1
11	RM152	GAAACCACCACACCTCACCG	60	CCGTAGACCTTCTTGAAGTAG	47.6
12	RM153	GCCTCGAGCATCATCATCAG	55	ATCAACCTGCACTTGCCTGG	55
13	RM21	ACAGTATTCCGTAGGCACGG	55	GCTCCATGAGGGTGGTAGAG	60
14	RM475	CCTCACGATTTTCCTCCAAC	50	ACGGTGGGATTAGACTGTGC	55
15	RM247	TAGTGCCGATCGATGTAACG	50	CATATGGTTTTGACAAAGCG	40
16	RM256	GACAGGGAGTGATTGAAGGC	55	GTTGATTTCGCCAAGGGC	55.5
17	RM259	TGGAGTTTGAGAGGAGGG	55.5	CTTGTTGCATGGTGCCATGT	50

Data analysis

Electrophoresed SSR gels were scored for the presence (1) or absence (0) of bands of molecular weight size in the form of the binary matrix for all the varieties studied. Data were analyzed to obtain Jaccard's coefficients among the isolates by using NTSYS-PC Version 2.1 software [12]. The SIMQUAL program was used to calculate the Jaccard's coefficients. A dendrogram was constructed using UPGMA (unweighted pair-group method with arithmetic averages) with the SAHN (sequential, agglomerative, hierarchical, and nested clustering) routine. Allele frequency, Genetic diversity and Polymorphism Information Content (PIC) values for each marker were analyzed using Power Marker software 3.25 [13].

Results and Discussion

Rice is the most important cereal crop that has been referred to as a global grain because of its use as a prime staple food in about 100 countries of the world. Most of the world's rice is cultivated and consumed in Asia, which constitutes more than half of the global population [7]. It is planted about 154 million hectares annually or on about 11 percent of the world's cultivated land. The rising demand, a saturation of cultivable fields and climate change cause a supply shortage of a crop in the future. By the near 2025, about 785 million tones of paddy which is 70 percent more than the current production will be needed to meet the growing demand. To achieve the target yield that is required to sustain the world population, rice varieties with a yield advantage of about 20 percent over currently grown varieties must be developed [14].

In the present investigation, initially, 17 primers were screened for amplification of DNA samples of rice varieties. All the 17 primers were amplified successfully and selected for varietal identification on the basis of a sharp and clear banding pattern. Further PCR reactions were carried out using 17 SSR primers. Selected 17 SSR primers amplified 34 different loci in all the samples. The band size of the amplified product ranged from 100bp-300bp. Maximum numbers of allele (3) were scored by the primers RM21 while minimum numbers of allele (1) were scored by the primers RM484. Out of these amplified loci, 33 were found to be polymorphic (94.11%). The average number of bands per primer was 2.00 while, the average number of polymorphic bands per primer was 1.94. Sixteen primers were able to amplify polymorphic alleles and only one primer i.e. RM484 found to be monomorphic (Table-3). PCR amplification gave a good and clear banding profile which has been obtained by the primers with 50 to 60% GC content. Brondani [15] observed an increasing GC content of the primer with an increased number of bands. The explanation for the correlation between GC content and the number of bands may be the stability of base

complementation of A with T. The amplified DNA profiling was scored according to the presence and absence of bands. Fritsch et al. [16] also reported the importance of the GC content of primers on the yield of PCR amplified products.

Table-3 SSR markers profile in rice varieties

S.N.	Primer	TB	PB	PP	PIC	Band size (in bp)	Allele Frequency	Genetic Diversity
1	RM474	2	2	100	0.71	140,160	0.4000	0.6775
2	RM413	2	2	100	0.71	160,180	0.3556	0.7230
3	RM484	1	0	0	0.00	200	1.0000	0.0000
4	RM19	2	2	100	0.72	250, 300	0.3333	0.7299
5	RM212	2	2	100	0.71	100, 150	0.5333	0.6044
6	RM249	2	2	100	0.73	140, 160	0.2889	0.7477
7	RM250	2	2	100	0.70	160, 180	0.4000	0.7457
8	RM280	2	2	100	0.79	180, 200	0.3556	0.7042
9	RM541	2	2	100	0.73	80, 100	0.4444	0.6064
10	RM11	2	2	100	0.70	180, 200	0.3778	0.6746
11	RM152	2	2	100	0.70	150, 200	0.5556	0.6153
12	RM153	2	2	100	0.72	200, 220	0.5556	0.5956
13	RM21	3	3	100	0.89	140, 180, 200	0.2667	0.8128
14	RM475	2	2	100	0.72	200, 230	0.3778	0.7605
15	RM247	2	2	100	0.73	160, 200	0.4889	0.7032
16	RM256	2	2	100	0.68	100, 150	0.6889	0.4770
17	RM259	2	2	100	0.75	160, 180	0.3778	0.6854
Total		34	33	-	-	-	-	-
Mean		2.00	1.94	94.11%	0.68	-	0.4588	0.6390

TB- Total Band, PB- Polymorphic Bands, PP- Percentage of Polymorphism, PIC-Polymorphism Information Content

According to Salgotta et al. [17] out of 52 SSR markers selected 41were found to be polymorphic while 11 were found to be monomorphic among parental lines. A total of 77 alleles were detected and the number of alleles per locus ranged from 1 to 6. Similar results were observed in rice [18,19,20]. During the present investigation similar type of result was obtained for varietal identification; all SSR markers were polymorphic except one.

According to Jaccard's similarity coefficient, the highest similarity 97% was found between MTU1011 and SONAMRICE and the lowest (25.7%) between KATKIRICE and CHANDRAHASNI. The range of Jaccard's similarity coefficient 25.7-97% indicates a higher level of diversity among rice varieties. To evaluate the Polymorphism information content (PIC) value of a marker the frequencies of each marker allele must be determined. Markers with greater numbers of alleles tend to have higher PIC values and thus are more informative [21]. For genetic diversity analysis, the PIC value was evaluated based on the specific locus/marker. The overall PIC value ranged between 0.0 (RM341) to 0.582 (RM256) with a mean value of 0.324 reported by Gour et al. [22]. Similar results were observed for evaluation of PIC value by many researchers such as [20,23,24]. In the present investigation, the highest PIC value (0.89) was observed in RM21 with 3 alleles among the 45 varieties. Primers RM280, RM259, and RM249 also exhibited higher PIC scores and a high number of alleles and the lowest PIC value was recorded in RM484 (0.00). Whereas the pic value ranged 0.00 (RM484) to 0.89 (RM21) with a mean value of 0.68 in different sets of rice varieties which were closer to the result as previous studies.

In UPGMA cluster analysis, the varieties were grouped into two clusters (Fig.1). The major group contained 44 varieties and the minor group contained only one variety KATKIRICE. The major group is further classified into two sub-groups 'A' and 'B'. Sub-group 'A' contained 37 varieties whereas sub-group 'B' contained 7

varieties. Variety KATKIRICE was found to be diverse from others and placed at end of the cluster. Similar clustering patterns have also been reported in rice [21,25,26].

Principle components analysis of 45 rice varieties was carried out according to the similarity coefficient. In this analysis, three groups were divided into group 'A', group 'B', and group 'C'. Group 'A' holds 26 varieties namely KARIKAMOL, KARIKAMOD, BASMATIP1460, IGKVR2, HATHWA, BAIJARRICE, SAMBLESHWARI, DANTESHWARI, JHIGAFULL, AMPORICE, IR81, SAHBAGHI, KRANTI, DUBRAJ, JR201, KATKIRICE, SATARISAFARI, BAMLESHWARI, CULTURERICE, SUKNANDAN, SONAMRICE, MTU1011, MTU1010, IR64, IGKVR1 AND CHANDRAHASNI and these varieties were placed closely due to more similarity among them. Group 'B' varieties has 16 varieties namely KANASHRI, IGKVR1244, SATHARHSAFRI, CHOTISAFRI, MAHAMAYA, SARNA, GHINMORI, HMT, GERAFULL, SAFADARNA, M2, 1010, WGL32100 AND BASMATIPS3 were placed closely with more similarity and group 'C' possessed five varieties namely LALURICE, RANIKAJAL, KARHANI, SURJARICE and CHINDEMORI were more diverse from other varieties (Fig.2). These five varieties were collected from same location.

Three-dimensional scaling of 45 rice varieties also revealed similarity conferring principal components. These varieties were divided into three groups namely group 'A', group 'B' and group 'C', group 'A' contained 24 varieties namely KRANTI, DUBRAJ, SAHBAGHI, JR200, DANTESHWARI, SANBLESHWARI, IR81, JHIGAFULL, GERAFULL AMPORICE, MAHAMAYA, 1010, SARNA, WGL32100, MTU1010, SUKNANDAN, BAMLESHWARI, CULTURERICE, BASMATIPS3, IR64, IGKRV1, CHANDRAHASNI, SONAMRICE and MTU1011 exhibiting high similarity amongst them. Group 'B' contained 17 varieties namely CHINDEMORI, KARIKAMOL, KARIKAMOD, BASMATIP1460, SAFADSRNA, M2, BAIJERRICE, SATARISAFRI, HMT, HATHWA, IGKVR1244, IGKVR2, GHINMORI, SATHARHSAFRI, KATKIRICE, CHOTISAFRI and KANASHARI. Group 'C' contained four varieties such as LALURICE, RANIKAJAL, KARHANI and SURJARICE (Fig.3). This grouping was similar to the phylogenetic tree (Fig. 1) as all of these four varieties were groups in the same group.

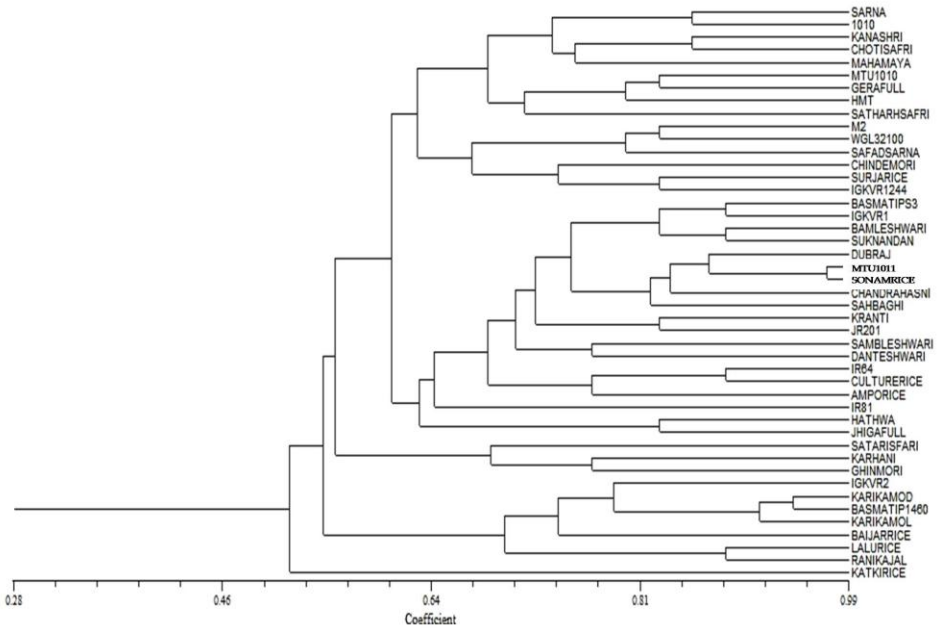

Fig.1. Phylogenic tree showing relationship among rice varieties based on SSR data

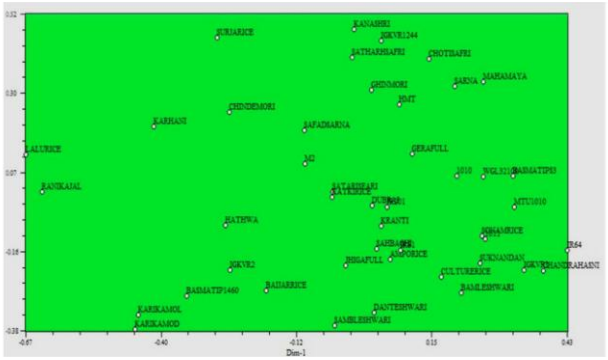

Fig.2. Two dimensional scaling of rice varieties based on Principal Component Analysis (PCA)

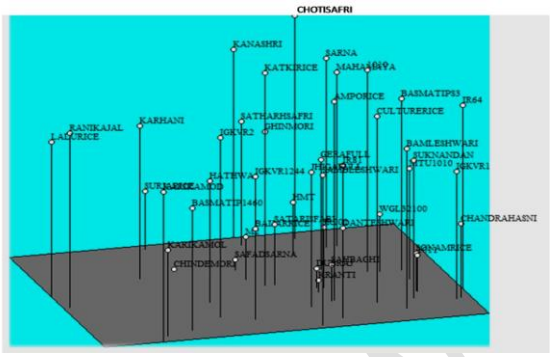

Fig.3. Three dimensional scaling of rice varieties based on SSR data

Among all the rice varieties selected for the present investigation, KATKIRICE showed higher genetic diversity from all other varieties as, it formed separate sub group.

Conclusion

The molecular marker technique used in this study has proved to be successful in elucidating relationships among the 45 rice varieties, in identifying species-specific markers and generating a fingerprinting key as the important resources for the breeding and management of rice germplasm/ varieties. In addition, some of these markers can be used for marker-assisted selection (MAS) for the genetic improvement of rice.

References

1. Tripathi S, Singh SK, Srivashtav V, Khaire AR, Vennela P, Singh DK. Molecular diversity analysis in rice (Oryza sativa L.) using SSR markers. Elect. J. Plant Breed., 2020, 11(03): 776-782.
2. Nachimuthu VV, Muthurajan R, Duraialaguraja S, Sivakami R, Pandian BA, Ponniah G, Gunasekaran K, Swaminathan M, Suji KK, Sabariappan R. Analysis of population structure and genetic diversity in rice germplasm using SSR markers: an initiative towards association mapping of agronomic traits in *Oryza sativa*. Rice. 2015, 8(1):1-25.
3. Dudley JW. Plant breeding- a vital part of improvement in crop yields, quality and production efficiency. In: Frey KJ, editor. Historical Perspectives in Plant Sciences. Ames: Iowa State University Press; 1994, p. 163–77.
4. Raza Q, Riaz A, Saher H, Bibi A, Raza MA, Ali SS. Grain Fe and Zn contents linked SSR markers based genetic diversity in rice. PLoS ONE 2020, 15(9): e0239739. https://doi.org/10.1371/journal.pone.0239739
5. Islam MR, Gregorio GB, Salam MA, Collard BCY, Singh RK and Hassan L. Validation of *SalTol* linked markers and haplotype diversity on chromosome 1 of rice. Molecular Plant Breeding. 2012,3(10): 103-114.
6. Syed MA, Iftehkaruddaula KM, Akter N, Biswas PS. Molecular Diversity Analysis of Some Selected BBRI Released Rice Varieties using SSR Markers. Int. Res. J. Biol. Sci., 2019.2: 51-58
7. Kaur Y, Kaur R, Khanna R, Sidhu N. Assessment of genetic diversity in a set of rice (Oryza sativa L) genotypes using molecular marker. J. Cereal Res., 2020, 12(3): 327- 333. http://doi.org/10.25174/2582-2675/2020/107380.
8. Kachare S, Tiwari S, Tripathi N, Thakur VV. Assessment of genetic diversity of soybean (*Glycine max* (L.) Merr.) genotypes using qualitative traits and microsatellite makers. Agri. Res., 2019, DOI: 10.1007/s40003-019-00412-y.
9. Tiwari S, Tripathi N, Tsuji K, Tantwai K. Genetic diversity and population structure of Indian soybean (*Glycine max* (L.) Merr.) as revealed by microsatellite markers. Physiol. Mol. Biol. Plants. 2019, 25(4):953-964.

10. Song JM, Arif M, Zi Y, Sze SH, Zhang M, Zhang HB. Molecular and genetic dissection of the USDA rice mini-core collection using high-density SNP markers. Plant Science, 2021, 308: 110910, https://doi.org/10.1016/j.plantsci.2021.110910.

11. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus, 1990, 12(1):13–15.

12. Rohlf FJ. NTSYSpc: numerical taxonomy and multivariate analysis system. 2000, version 2.1. Exeter Software, NY.

13. Liu K, Muse S. Power marker: new genetic data analysis software. 2005, Release 3.0. http://powermarker.net; verified 9 September.

14. Chakravarthi BK, Naravaneni R. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). Afr. J. Biotechnol., 2006, 5(9): 684-688

15. Brondani C, Borba TCO, Rangel PHN and Brondani RPV. Determination of genetic variability of traditional varieties of Brazilian rice using microsatellite markers. Genet. Mol Biol., 2006.29(4): 676-684.

16. Fritsch P, Hanson MA, Spore CD, Pack PE and Rieseberg LH. Constancy of RAPD primer amplification strength among distantly related taxa of flowering plants. Plant Mol. Biol. Rep., 1993, 11(1): 10-20.

17. Salgotra RK, Gupta BB, Bhat JA, Sharma S. Genetic Diversity and Population Structure of Basmati Rice (*Oryza sativa* L.) Germplasm Collected from North Western Himalayas Using Trait Linked SSR Markers. PLoS ONE, 2015, 10(7): e0131858. https://doi.org/10.1371/journal.pone.0131858.

18. Sundaram RM, Kumar NB, Biradar SK, Balachandran SM, Mishra B, Ilyas AM, Viraktamath BC, Ramesha MS, Sarma NP. Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. Euphytica. 2008, 163(2): 215–224.

19. Nandakumar N, Singh AK, Sharma RK, Mohapatra T, Prabhu KV, Zaman FU. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. Euphytica. 2004, 136(3): 257-264.

20. Seetharam K, Thirumeni S, Paramasivam K. Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers and morphological characters. Afr. J. Biotechnol., 2009. 8(10): 2050-2059.

21. Meti N, Samal KC, Bastia DN, Rout GR. Genetic diversity analysis in aromatic rice genotypes using microsatellite based simple sequence repeats (SSR) marker. Afr. J. Biotechnol., 2013, 12(27): 4238-4250.

22. Gour L, Koutu GK, Singh SK, Mishra DK. Genetic potency identification of indigenous rice (*Oryza sativa* L.) lines using quality assessment and SSR marker analysis. Int. J. Chem. Stud., 2016, 5(4): 97-101.

23. Singh D, Kumar A, Sirohi A, Kumar P, Singh J, Kumar V. Improvement of Basmati rice (*Oryza sativa* L.) using traditional breeding technology supplemented with molecular markers. Afr. J. Biotechnol , 2011, 10(4): 499-506.

24. Shrivastava A, Mishra DK, Koutu GK, Singh SK, Prakash V, Sohgaura NE. Assessment of genetic diversity of restorer and maintainer lines of rice using SSR markers. The Ecoscan. 2015, 9(1&2): 265-269.

25. Kunusoth K, Vadivel K, Sundaram RM, Sultana R, Rajendrakumar P, Maganti S, Subbarao LV, Chin-Wo SR. Assessment of genetic diversity of elite indian rice varieties using agro-morphological traits and SSR Markers. J. Exp. Agri. Int., 2015, 6(6): 384-401.

26. Vhora ZA, Trivedi RU, Chakraborty SU, Ravikiran RA, Sasidharan N. Molecular studies of aromatic and non-aromatic rice (*Oryza sativa* L.) genotypes for quality traits using microsatellite markers. The Bioscan, 2013, 8(2):359-362.