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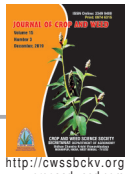

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Genetic diversity assessment of rice using SSR markers

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ABSTRACT

Rice is the world's most significant cereal crop. It is always desirable to enhance the yield and improve the quality of rice in order to meet the demands of the world's population. One of the basic criteria for genetic up-gradation of rice varieties is the exploration of gene pool divergence in current rice germplasm. In this study, genetic diversity assessment was performed to find the variations among 27 rice genotypes with the help of seven polymorphic Simple Sequence Repeat markers. The ranges of Polymorphic information content (PIC) value and effective allele per locus of microsatellite were 0.431-0.7435 and 1.757-3.898 respectively. Based on the constructed dendrogram on NTSYS-pc Version 2.20 program, the genotypes were grouped into two clusters having two sub clusters in each group. In future breeding program, this analysis of genetic diversity will be effective for selecting desirable rice genotypes as a donor by breeders.

***Keywords*:** Effective allele per locus, genetic diversity, polymorphic information content, rice, simple sequence repeat.

Rice (*Oryza sativa*) is a self-pollinated crop that belongs to the Poaceae family. Rice is the primary food crop for most of the Asian countries and it also feeds more than half of the world's population (Khush, 2005). Approximately, 20% of the world's dietary energy supply is provided by rice, so it is consumed in major portions of the daily diet. The grain of rice mainly contains starch, water and protein. In rice grains, several minerals like calcium, phosphorus, magnesium, iron, copper, manganese, zinc and vitamins are also found (Oko *et al*., 2012). Whole rice grains are used for cooking and consumption, so it is very much desirable to improve the quality of rice grain. Along with improving quality, raising paddy yield, increasing nutrient content, and producing multi-stress resistant rice varieties are also required to meet global demand (Ali *et al*., 2018).

The rice plant is adapted and cultivated among different regions in the World, so diversity in different rice varieties can be observed. Though, Asian rice (*Oryza sativa*) is one of the rice species which is mostly used for cultivation under genus *Oryza*, but it was also differentiated into six groups including *Oryza sativa indica* and *Oryza sativa japonica* (Kaur *et al*., 2015). Genetic diversity refers to the differences in genetic information between and within individuals in a population. So, analysis of genetic diversity is fundamental for the betterment of the plant species (Pandey *et al*., 2019). For registering as a new rice variety, it is crucial to perform "Distinctness", "Uniformity" and "Stability" (DUS) test among different rice varieties which can be performed on the basis of agro-morphological, biochemical, physiological and molecular approaches (Sarao *et al*., 2010). Among all of these approaches, diversity analysis based on molecular markers is most appropriate, because there are no environmental or epistatic effects and analysis requires less time (Ye-yun *et al*., 2005). The rice genome is already sequenced for which genomic studies can be performed with precision and DNA based markers can be utilized for diversity analysis. The most widely used molecular markers of rice for genetic diversity analysis are RAPD (Random Amplified Polymorphic DNA) (Dominant in nature), AFLP (Amplified Fragment Length Polymorphism) (Dominant in nature), RFLP (Restriction Fragment Length Polymorphism) (co-dominant in nature), ISSR (Inter Simple Sequence Repeat) (Dominant in nature), and SSR (Simple Sequence Repeat) (co-dominant in nature), with SCAR (Sequence Characterized Amplified Region) (co-dominant in nature) and SNP (Single Nucleotide Polymorphism) (co-dominant in nature) as the others (Saini *et al*., 2004; Kaur *et al*., 2015).

Simple Sequence Repeats (SSRs) are the tandem repetitive sequences of short DNA motifs which are 1-6 base pair (bp) in length and co-dominant in nature

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(Cheng *et al*., 2016). The primers that surround these types of areas can be used to amplify these markers. SSR markers have been observed to have greater polymorphism than most of the other molecular markers, making the genetic diversity study with SSR markers more preferred (Ye-yun *et al*., 2005).

The purpose of this research was to utilize seven different SSR markers for investigating the genetic diversity in 27 rice genotypes. Analyzing the genetic diversity helps to figure out which genotypes are genetically distinct from one another so that a crossing program between genotypes from different clusters can be performed and this will increase the chances of desired recombinants in segregating generations.

MATERIALS AND METHODS

All of these experiments were performed at Integrated Rural Development and Management (IRDM) faculty centre, RKMVERI, Narendrapur in 2019.

Experimental materials

Seeds of twenty-seven rice genotypes (Table 1) were taken from the Department of Genetics and Plant Breeding, IRDM faculty centre, Narendrapur, RKMVERI and used as experimental materials. Seeds were germinated at 25 °C in BOD incubator (YOMA, Mfg by Indian Instruments Manufacturing Co., Kolkata). After the germination, the seeds were planted in small pots where the mixture of soil and cocopeat was already added in 1:1 ratio. The rice plants were grown in green house.

Genomic DNA isolation

Extraction of genomic DNA of individual rice genotypes was performed at the three leaves stage using CTAB (Cetyle Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1987). The DNA samples were run on 0.8% agarose gel at 80V for 40 min. The DNA bands were observed on the gel documentation system (BIO-RAD). The concentrations of the DNA samples were checked by using Nanodrop machine (Thermo SCIENTIFIC). The isolated DNA samples were stored at -20 °C.

Genomic DNA amplification

Fifteen rice microsatellite markers denoted by RM (Rice Microsatellite) and covering 12 chromosomes were initially selected from the Gramene website (www.gramene.org) and evaluated for polymorphism. Based on the polymorphisms, seven microsatellite markers were further utilized for diversity analysis (Table 2). In a thermocycler (Eppendorf), the isolated genomic DNA samples from individual rice genotypes were amplified by Polymerase Chain Reaction (PCR). The PCR was conducted in a reaction solution of 25 µl prepared in a PCR tube containing 50 ng isolated rice genomic DNA, 2.5 µl of 10X PCR buffer [Taq buffer, composition- Tris (pH 9.0), KCL, Gelatin], 0.2 µmol L^{-1} of each Forward and Reverse primer, 2 µl $MgCl_2$ (25 mM), 0.2 mM dNTP mix and 0.2 µl Taq polymerase (3 U/µl). The cycle profile of the thermocycler was preheating at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, primer annealing at a specific temperature for a particular primer pair at 30 seconds, primer extension at 72°C for 1 minute and final extension at 72°C for 5 minutes. After amplifications, the PCR products were separated on 2% agarose gel with 50 bp and 100 bp DNA ladders. The observations of bands were carried out using the gel documentation system.

Data analysis

The presence or absence of a particular allele in a genotype was used to provide a score. The allele was assigned as a value of 1 if it was present and a value of 0 if it was absent. The data was then used to create a 1/0 matrix. The genotypes were divided into groups according to the allelic size of the PCR products. The Dendrogram was created using the NTSYS-pc Version 2.20 program. The microsatellite loci's Effective allele per locus (Aep) and polymorphic information content (PIC) were computed.

The formula for calculating the Aep

$$Aep = 1/(1-Hep)$$

Where Hep= the genetic diversity for individual locus and Hep is equal to $1-\Sigma P^2i$, Pi means the frequency of i^{th} allele at the locus. (Pandey *et al*., 2015).

The formula for calculating the PIC value of microsatellite marker:

$$PIC_i = 1 - \Sigma P_{ij}^2$$

Where i= 1 to n and P_{ij}= the frequency of j^{th} allele for the i^{th} band scored for a specific marker (Goswami *et al.*, 2017).

RESULTS AND DISCUSSION

Although 15 SSR markers were used in the current investigation to disclose the genetic background of twenty-seven rice genotypes, only seven polymorphic markers were chosen for genetic diversity analysis. The seven polymorphic SSR markers were RM152, RM207, RM1385, RM257, RM263, RM25181, and RM247. In case of most of the primers, one strong band could be seen after running the PCR amplified products on agarose gel [Fig. 1 (A), Fig. 1 (B)]. These markers were seen to generate polymorphic bands in the population, indicating that there was variation. The number of alleles ranged from 3 to 5 (Table 3). PIC

Table 1: List of rice genotypes for present experiment

Sl. No.	Name of the genotype	Origin
1.	ARC 10086	Assam
2.	Mali 4	Mali Agri tech, Ranaghat
3.	Kasalath	India
4.	IRBB 59	IRRI
5.	MTU 1010	ANGRAU, AP
6.	Swarna	IRRI collab CRRI
7.	Dular	Landrace
8.	IR 24	IRRI
9.	Azucena	Philippines
10.	Swarna Sub-1	IRRI
11.	Samba Mahsuri	Acharya N G Ranga Agricultural University (ANGRAU)
12.	Lemont	Philippines
13.	Restorer Line-51 (R-51)	Mali Agri tech, Ranaghat
14.	CN1646-2	Chinsurah, West Bengal
15.	Sabita	Landrace
16.	Ratna	India
17.	Restorer Line (R-71)	Mali Agri tech, Ranaghat
18.	Patharea	Thane, Maharashtra
19.	*Indica Javanica* (TC-25-2-1)	Mali Agri tech, Ranaghat
20.	*Indica Javanica* (TC-26-2-1)	Mali Agri tech, Ranaghat
21.	Nipponbare	Japan
22.	IR 64	IRRI
23.	IR 68144-2B-2-2-3-1-127	IRRI
24.	Kalinga-2	CRRI, Cuttack
25.	Danaguri	Local Landrace, West Bengal
26.	Zheshan-2	China
27.	ARC 100372	Assam

Table 2: Primers for the genetic diversity analysis

Name of markers	Chromosome	Repeat Motif	Sequences of the forward primers	Sequences of the reverse primers	Annealing Temperature (°C)
RM152	8	(GGC)10	GAAACCACCACA CCTCACCG	CCGTAGACCTTCTT GAAGTAG	55
RM247	12	(CT)16	TAGTGCCGATCGA TGTAACG	CATATGGTTTTGACA AAGCG	55
RM1385	2	(AG)39	ATGACAGGTAAGG TGTGGTG	TGAACATCATCT TCGAATCC	55
RM207	2	(CT)25	CCATTCGTGAGA AGATCTGA	CACCTCATCCTCG TAACGCC	55
RM263	2	(CT)34	CCCAGGCTAGCTC ATGAACC	GCTACGTTTGA GCTACCACG	
RM257	9	(CT)24	CAGTTCCGAGCA AGAGTACTC	GGATCGGACGT GGCATATG	55
RM25181	10	(TTC)22	AAAGAGCTTCCC TAATGGCTT	GAGAGAATGACCT CTCCCAAG	58.4

Table 3: Characteristics of polymorphic SSR markers of present study

No	Name of the marker	No of alleles	Aep	PIC
1	RM152	5	3.898	0.7435
2	RM247	3	1.923	0.48
3	RM1385	3	2.024	0.506
4	RM207	4	1.757	0.431
5	RM263	3	2.841	0.648
6	RM257	4	3.279	0.695
7	RM25181	5	2.906	0.656
MEAN			**2.661**	**0.594**

Table 4: Cluster composition of 27 rice genotypes on the basis of seven SSR markers

Cluster	Sub cluster	Name of the genotype
CL1	CL1-A	IR64
	CL1-B	*Indica Javanica* (TC-26-21), Azucena, Swarna Sub-1
CL2	CL2-A	Mali-4, Lemont
	CL2-B	Dular, IR24, Swarna, Kalinga-2, Samba Mahsuri, Zheshan-2, R-51, CN1646-2, Sabita, Ratna, R-71, Patharea, *Indica Javanica* (TC-25-2-1), MTU1010, Nipponbare, IR68144-2B-2-2-3-1-127, Danaguri, ARC-100372, IRBB59, Kasalath, ARC-10086

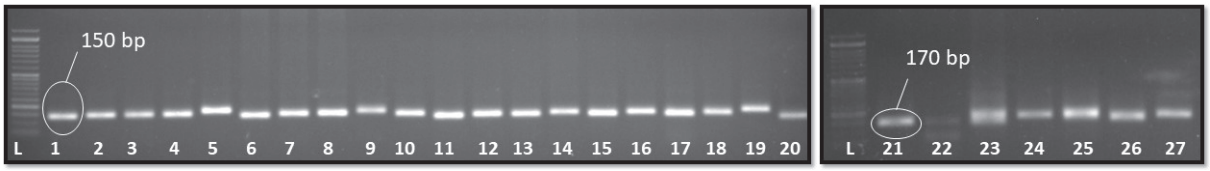

Fig. 1 (A): Electrophoregram of 27 different rice genotypes (genotype no: 1-27, as described in Table 1) using RM25181 primer where L= 50bp DNA ladder

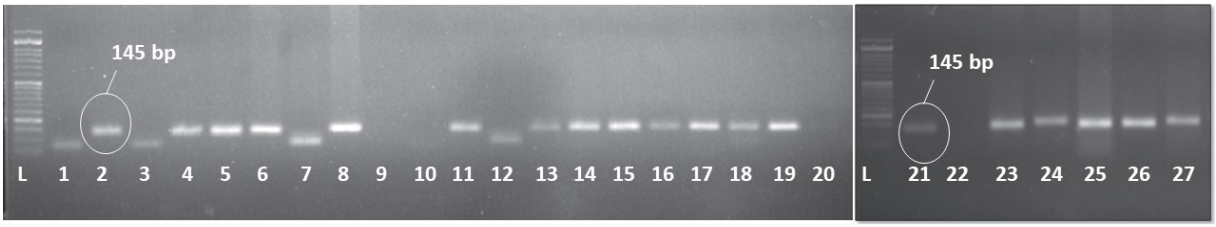

Fig. 1 (B): Electrophoregram of 27 different rice genotypes (genotype no: 1-27, as described in Table 1) using RM207 primer where L= 50bp DNA ladder

values of 5 primers including RM152, RM1385, RM263, RM257 and RM25181 were more than 0.5, where RM152 had the highest PIC value (Table 3). The average PIC value was 0.594, so it signified that most of the SSR loci were highly polymorphic. It was previously reported that if the PIC value is more than 0.5, then the polymorphism can be defined as high for the locus (Botstein *et al*., 1980). Mukherjee *et al*.(2013) characterized 20 rice genotypes with the help of SSR markers and they also reported that the primers-RM25181 and RM257 were highly polymorphic which was also found in this study. Here the range of Aep was from 1.757-3.898. Both the PIC and Aep value was highest for RM152 primer and lowest for RM207 primer.

Using NTSYS-pc Version 2.20 software, it was observed that 27 rice genotypes consisted two clusters which were formed with coefficient- 0.81 (Fig. 2) (Table 4). The first cluster consisted 4 genotypes namely, IR64, TC 26-2-1, Azucena and Swarna sub-1. The genotype IR64 was in cluster 1-A and rest 3 genotypes were in cluster 1-B. The second cluster was also composed of two sub clusters. The genotypes Mali-4 and Lemont constituted in cluster 2-A while 21 genotypes were grouped in cluster 2-B. In a study of genetic diversity of rice, it was previously reported that the genotypes

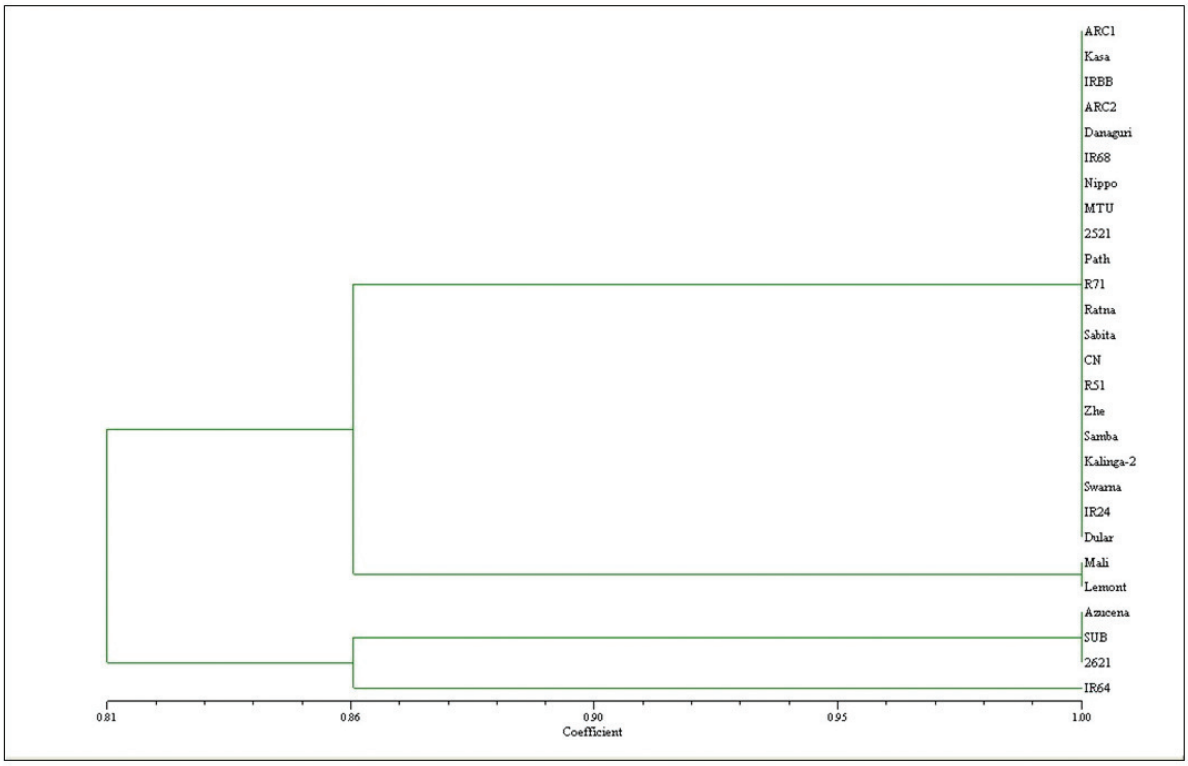

Fig. 2: Dendrogram of 27 different rice genotypes using NTSYS-pc Ver 2.20. Here ARC1= ARC 10086, Kasa= Kasalath, IRBB= IRBB59, ARC2= ARC 100372, IR68= IR68144-2B-2-2-3-1-127, Nippo= Nipponbare, MTU= MTU1010, 2521= TC 25-2-1, Path= Patharea, CN= CN1646-2, Zhe= Zheshan-2, Samba= Samba Mahsuri, SUB= Swarna sub-1, 2621= TC 26-2-1.

ARC-10086 and Lemont belong to one cluster and the genotypes Zheshan-2 and Nipponbare belong to the same cluster (Nath *et al*., 2021), where in this study, Zheshan-2, Nipponbare and ARC-10086 were grouped into a single cluster as well as in the same sub cluster (cluster 2-B) whereas Lemont were grouped into the same cluster with Zheshan-2, Nipponbare and ARC-10086 but different sub cluster (cluster 2-A).

Furthermore, cluster analysis was used to classify genotypes representing two distinct subgroups, revealing that significant variation can be predicted even within each subpopulation. The clustering pattern clearly showed that some genotypes from diverse geographic origins were grouped together in the same cluster or some genotypes from same geographic areas did not come in the same cluster. The results are also in conformity with the findings of Bhattacharjee *et al.* (2019). When choosing parents for hybridization, it is preferable that the parents come from different clusters because a cross between two distinct parents with the greatest genetic variation or pertaining to a widely variable group, as a transgressive segregant in the filial generation is desired (Dasgupta and Das, 1987).

The diversity study assisted in the generation of information on various groups as well as the identification of various genotypes as potential donors for future breeding operations. Using the molecular markers which were used to analyze the population; the genotypes were correctly classified on the basis of their genetic background. As a result, the SSR markers utilized in this work can be used to assess genetic variation in future breeding attempts.

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REFERENCES

Ali, J., Jewel, Z.A., Mahender, A., Anandan, A., Hernandez, J. and Li, Z. 2018.Molecular genetics and breeding for nutrient use efficiency in rice. *Int. J. Mol. Sci.*, **19** : 1-27

Bhattacharjee, M., Iqbal, A., Singha, S., Nath, D., Sh. Prakash and Dasgupta. T. 2019.Genetic diversity in *Sesamum indicum* L. *Bangladesh J. Bot.,* **48** : 497-506

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980.Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, **32** : 314-331

Cheng, J., Zhao, Z., Li, B., Qin, C., Wu, Z., Trejo-Saavedra, D.L., Luo, X., Cui, J., Rivera-Bustamante, R.F., Li, S. and Hu, K. 2016.A comprehensive characterization of simple sequence repeats in pepper genomes provides valuable resources for marker development in Capsicum. *Sci. Rep.,* **6** : 1-12

Dasgupta, T. and Das, P.K. 1987.Inheritance of pod length and cluster number in blackgram (*Vigna mungo* L.). *Ind. J. Agril. Sci.*, **57** : 50-52

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

Goswami, S., Kar, R.K., Paul, A. and Dey, N. 2017.Genetic potentiality of indigenous rice genotypes from Eastern India with reference to submergence tolerance and deepwater traits. *Curr. Plant Biol*., **11-12** : 23-32.

Gramene. www.gramene.org (Last accessed on 30 January, 2019).

Kaur, S., Panesar, P.S., Bera, M.B. and Kaur, V. 2015.Simple sequence repeat markers in genetic divergence and marker-assisted selection of rice cultivars: a review. *Crit. Rev. Food Sci. Nutr.*, **55** : 41-49.

Khush, G.S. 2005.What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biol.*, **59** : 1-6.

Mukherjee, B., Das, P., Alam, Q., Nath, D. and Dasgupta, T. 2013. Molecular characterization of rice genotypes using microsatellite markers. *Oryza*, **50** : 35-40

Nath, A., Nath, D. and Dasgupta, T. 2021. Population structure and diversity analysis of Indica and Japonica rice genotypes. *Bulgarian J. Agric. Sci.*, **27**:120-127.

Oko, A.O., Ubi, B.E., Efisue, A.A. and Dambaba, N. 2012.Comparative analysis of the chemical nutrient composition of selected local and newly introduced rice varieties grown in Ebonyi State of Nigeria. *Int. J. Agric. For.,* **2** : 16-23.

Pandey, A., Khan, M.K., Isik, R., Turkmen, O., Acar, R., Seymen, M. and Hakki, E.E. 2019. Genetic diversity and population structure of watermelon (*Citrullus* sp.) genotypes. *Biotech*, **9** : 1-14

Pandey, S.K., Das, A., Rai, P. and Dasgupta, T. 2015.Morphological and genetic diversity assessment of sesame (*Sesamum indicum* L.) accessions differing in origin. *Physiol. Mol. Biol. Pl.*, **21** : 519-529.

Saini, N., Jain, N., Jain, S. and Jain, R.K. 2004.Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica*, **140** : 133-146.

Sarao, N.K., Vikal, Y., Singh, K., Joshi, M.A. and Sharma, R.C. 2010.SSR marker-based DNA fingerprinting and cultivar identification of rice (*Oryza sativa* L.) in Punjab state of India. *Plant Genet. Resour.,* **8** : 42-44.

Ye-yun, X., Zhan, Z., Yi-ping, X. and Long-ping, Y. 2005. Identification and purity test of super hybrid rice with SSR molecular markers. *Rice Sci*., **12** : 7-12.