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Analysis of Genetic Diversity in Rice (O*ryza Sativa* L.) cultivars using SSR Markers

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ABSTRACT

Rice (Oryza sativa L.), mostly grown staple food crop which had a pivotal role in the Indian civilization and provides economical, food security for more than 150 million households. In the context to importance of this crop, the diversity of Oryza sativa has attracted great interest. The availability of an array of molecular marker systems allowed comparing the efficiency of two of these marker systems to estimate the relationships among various taxa. Therefore present study was intended to analyze genetic relationship in rice using SSR markers. Experiment was conducted in randomized complete block design manner and twenty rice cultivars were transplanted during kharif season. Physiological observations were recorded as per need. Total genomic DNA was isolated collecting fresh leaves by random selection in each plot of twenty germplasm lines from each replication using CTAB method. Isolated DNA quality was determined by UV-VIS spectrophotometer and integrity was confirmed using agarose gel electrophoresis. PCR amplification was carried out using ten SSR markers. Amplified PCR products were gel electrophoresed which shows total of 159 bands out of which 30 bands are polymorphic and 129 where monomorphic. The highest genetic similarity was found between IR78537-32-1-2-1 and IR7736-54-3-1-2, WAS197-B-6-3-12, IR78537-32-1-2-1, WAS197-B-6-3-12, IR7736-54-3-1-2, and WAS197-B-6-3-12, Sarjoo-52 and IR78537-32-1-2-1, while least genetic similarity between Kalanamak and IR77734-93-2-3-2.

Keywords: *Rice, Genetic diversity, SSR Markers.*

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INTRODUCTION

Rice (*Oryza sativa* L.), the important food crop occupies a pivotal role in Indian agriculture and consumed as a primary food source for more than a one third of world's population. It is grown in about 154 million hectares annually on about 11% of the world's total cultivated land with a production of 600 million tones. Economic survey of India 2013-14 represents largest area and second highest production in the world. The rice is thought to have been originated south and southeast tropical Asia, in a broad belt extending from north eastern India to Across Burma, Thailand Laos, and southern china. In this area, the greatest diversity of the cultivated native forms may be found. Based on morphological and physiological characteristics and on geographic adaption, these have been grouped into three more or less distinct ecological forms; tropical (India), temperate (japonica), and intermediate (javanica), [1, 2, 9]. In the past, breeders exploited plant species for their livelihoods that resulted in domestication of many of them as improved cultivars to produce food for the better supply of the human diet [19, 23]**.** Knowledge of the

genetic diversity of germplasm collections is an important foundation for crop improvement. Due to the importance of rice as a major world crop, the diversity of *Oryza sativa* has attracted great interest.
Conventional breeding approaches were used to improve rice cultivars, but the progress was very slow due to the time-consuming process, quantitative nature of most agronomic traits and difficulties in genotype selection (www.fao.org/docrep/004/y3557e/y3557e09.html). Diversity analysis in a landrace collection is very important for identifying new genes and further improvement of the germplasm [6, 34, 20]**.** In spite of the richness of genetic resources, only a small proportion of the world rice germplasm collections have been utilized in breeding programs, as a consequence a high genetic similarity is found within several commercial rice germplasms around the world [11]. There are several ways for estimation of diversity in germplasm, such as evaluation of phenotypic variation, biochemical and DNA polymorphisms. However, both phenotypic and biochemical characterizations are unreliable because they are environmentally challenged, labour demanding, numerically and phenologically limited. The DNA-based molecular markers are ubiquitous, repeatable, stable and highly reliable for the study of diversity [10, 30, 35]. Several classes of available DNA markers, microsatellite or simple sequence repeat (SSR) markers are considered the most suitable due to their multiallelic nature, high reproducibility, codominant inheritance, abundance and extensive genome coverage. A large number of SSR markers have been developed and mapped in rice, which vary in the degree of polymorphism depending on their position in the coding or noncoding segments, nature of their repeat motifs and the genome wide abundance [32, 16]**.** An ideal set of SSR markers providing genome wide coverage will facilitate an unbiased assay of genetic diversity which in turn will provide a robust, unambiguous molecular description of rice cultivars. The use of random markers for assessing genetic diversity might not reflect the functionally useful variations prevalent at the coding regions of the genome [36-38]. DNA based comparison between improved cultivars and ancestral wild types of rice preserved in gene banks indicated that improved cultivars (both *Indica* and *Japonica*) represented only about 15% of total available variability [38, 31]. The land races preserved in gene banks, though are rich in characters of value are not fully exploited for crop improvement. DNA fingerprinting techniques provide authentic characterization of cultivar and quantitative estimation of genetic diversity. More recently molecular markers, such as SNPs and simple sequence repeats (SSRs), which are genetically linked to fragrance and have the advantage of being inexpensive, simple, rapid and only requiring small amounts of tissue, have been developed for the selection of fragrant rice. . In rice, SSR markers have been effectively utilized for many purposes like genetic diversity and relatedness [21, 38] and maker assisted selection [33]. Recently molecular marker based available techniques seem to have potential to detect genetic differences within and among commercial cultivars [25, 5] serve the useful purpose.

MATERIAL AND METHODS

The present study was conducted during Kharif season of 2014 at the field laboratory and experiment station, Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.), India. Nursery of twenty rice genotypes (table 1) was sown in month of June. Experiment was conducted in randomized complete block design manner and two days old seedlings were transplanted into field after 28 days. During the experiment from each plot of twenty genotypes, three competitive plants were randomly selected from middle of the row in each replication and fresh leaves collected. Total genomic DNA was isolated from collected leaves using CTAB method [17] with some modification and RNase treatment was carried out for purification. Isolated DNA was quantified by UV-VIS spectrophotometer (Systronics, India). The samples having optical density between 1.8- 2.0 at A_{260}/A_{280} were selected and integrity of DNA was confirmed by 0.8 % agarose gel electrophoresis. Polymerase chain reaction for SSR amplificaton was conducted using ten anchored SSR primers synthesized from IDT Genie as per the method Zietkiewics *et al*. [39]. Amplification reaction for SSR (table 2) was performed in a total volume of 10µl using 1X Taq buffer, 2.5mM $MgCl_2$, 1mM dNTPs mix, 10µM forward, 10µM reverse primer, 0.5U/µl Taq polymerase, 5ng of template DNA and final volume by nuclease free water. All of the reagents used for PCR amplification were occupied by Genei Pvt. Ltd., Banglore. PCR thermal profiles initial denaturation at 94°C for 4 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min upto 35 cycles and final extension at 72°C for 7 min were found to be best for amplification. PCR products were analysed by 1.5% agarose gel electrophoresis and visualized with Alfa Innotech (Alphaimager) system. Jaccard's similarity coefficient was used to estimate genetic divergence. Each SSR marker, polymorphism information content (PIC) was determined as per the method of Senior *et al*. [26]. the PIC value ranging from '0' (Monomorphic) to '1' (highly discriminative with many alleles in equal in equal frequency) is an indication of discriminitative power of marker, not only for number of alleles at a locus but also for relative frequencies of those allele in the cultivar under study.

Table: 1 List of different rice cultivars lines used in present ivestigation

Sl.No.	Variety	Sl.No.	Variety
1.	TOX3440-17-1-1-1-1-1	12.	Kalanamak
2.	WAS 99-84	13.	WAS 515-B-10A-1-12
3.	IR77734-93-2-3-2	14.	WAS-197-B-4-1-22
4.	WAS-272-BB5-H5	15.	IR78006-55-2-3-3
5.	WAS-197-B-6-3-16	16.	IR78554-145
6.	IR78537-32-1-2-1	17.	Madhukar
7.	IR7736-54-3-1-2	18.	Sarjoo-52
8.	WAS 197-B-6-3-12	19.	Sukhsamrath B-15
9.	Jalmagna	20.	Swarna
10.	NDR-359		
11.	Swarna Sub-1		

RESULTS AND DISCUSSION

Rice (*Oryza sativa* L.) is the principal source of food more than a one third of the population and one of the most widely grown crops 40% of our food grain production, dedicated to rice. India is the prominent rice growing country accounting for about 20% of all world rice production. India is home to wide varieties of rice cultivars land researchers and many lesser non varieties as local entrepreneurs. As a major cereal crop, it is one of the most diversified crop species due to its adaptation to wide range of geographical, ecological and climatic regions including is rich in diversity including cultivars, landraces, wild and weedy relatives. The rice germplasm is rich reservoir of valuable genes that plant breeder's exploit it for crop improvement. DNA primers have the ability to differentiate different rice genotypes based on the differences in their genomic region and their number of alleles. Genetic diversity is key factor for germplasm conservation, characterization and breeding effects. In this study ten Microsatellite (SSR) markers which were used to assess the genetic diversity of twenty genotypes of rice cultivars. The results indicated significant variation among the rice genotypes. Microsatellites used to assess and identify number of alleles that were shared among the rice varieties relationship between the genotypes. Primer let to amplication (SSR) of total 159 bands out of which 30 bands are polymorphic and 129 were found to be monomorphic. Maximum number of bands obtained by Primer RM (1095) and RM (1075) while, minimum number of bands were amplified by primer RM (1093) and RM (1083) and average number of 15, 9 primer. Primer pair RM 1095 and RM 1075 with an average number of polymorphic PIC value (table 3) according to the analysis of Primer values 0.90, 0.88 each maximum for SSR markers. Highest PIC value observed for the SSR primer RM 1068 (0.097), RM 1092 (0.097) and RM 1095 (0.87). PIC value is reflection of allelic diversity and frequency among the cultivars. The observed pattern was considered with the finding of [15, 36]. The average PIC value of SSR loci was (0.417). The number of microsatellite marker varied from 2 to 3 on average of 15.9 allele per locus [13, 37] using the different genotypes [6, 29, 20, 11, 34, 21]. The Quantradiction among reports might due to the genotypes used and selection of microsatellite markers with scorable alleles one marker yielded two alleles while other markers produce single bands respectively. The SSR data was sterilized for estimating pair was genetic similarity among varieties using Jaccard's coefficient [12] method. The genetic similarity matrix was form the analysis using (UPGMA) Clustering elongation software programme (NTSYS) version 2.02E for dendogram cluster cultivar differentiation the degree of genetic relation among rice genotypes. The highest genetic similarity (1.00) measured between IR78537-32-1-2-1 and IR7736-54-3-1-2, WAS197-B-6-3-12, IR78537-32-1-2-1, WAS197-B-6-3-12, IR7736-54-3-1-2, and WAS197-B-6-3-12, Sarjoo-52 and IR78537-32-1-2-1. While least genetic similarity (between Kalanamak and IR77734-93-2-3-2) multivariant analysis based upon the genetic similarity data group all the 20 cultivars. The dendogram derived from the analysis was depicted as it contain four major clusters-I, II, III and IV comprised of 9, 5, 5, 1 cultivars. Out of the 5 clusters, cluster I consist of 8 genotypes, cluster II consist of 3 genotypes, cluster III consist of 2 genotypes, cluster IV consist of 8 genotypes, where as cluster V constitute of 2 genotypes based on present investigation, it was concluded that in general, there was parallelism between genetic and geographic diversity. This view point has been supported by the work of Babu *et al.,* [3], Roy *et al.,* [24], Bhutia *et al.,* [4], Singh *et al.,* [28], Chandra *et al.,* [7], Reddy *et al.,* [22], Sharma and Koutu, [27]. First major cluster consist of nine cultivars viz., TOX 3440-17-1-1-1-1-1, IR78537-32-1-2-1, IR7736-54-3-1-2, WAS197-B-6-3-12, Jalmagna, Sarjoo-52, WAS-515-B-10A-1-12, Madhukar and Sukhsamrath B-15. Group (Second major cluster) consist of five cultivars, viz. WAS-197-B-6-3-16, Swarna, WAS-197-B-4-1-22, Swarna Sub-1 and Kalanamak. Third major cluster consist of five cultivars viz. WAS 99-84, WAS-272-BB5-H5, NDR-359, IR78006-55-2-3-3 and IR78554-145. Fourth major cluster consist of single cluster viz. IR77734-93-2-3-2. In cluster I which is

comprised of nine cultivars showed maximum similarity (Similarity coefficient, 0.80) followed by cluster II which is consist of five cultivars at the Jaccard's coefficient 0.70 (70%) similarity coefficient cluster III which have seven cultivars group at the Jaccard's coefficient 0.44 (40%) similarity coefficient cluster IV which is consist of single genotypes they have not grouped to other cultivars, they are more distinct less similar in comparison to other varieties they are more distinct and diverse cultivar. In cluster I based on similarity coefficient cultivars, nine TOX 3440-17-1-1-1-1-1 and Sukhsamrath B-15 where distinctly located member of the cluster while IR78537-32-1-2-1 and IR7736-54-3-1-2 showed maximum similarity (0.96%) similarity. In cluster II WAS-197-B-6-3-16 and Swarna had maximum similarity (88%). WAS-197-B-6-3-16 and Kalanamak showed more distinctly located with only (60%) similarity. In case of cluster III WAS 99-84 and WAS-272-BB5-H5 is more similar showed 0.88 (88%) similar, while other members had less similarity. Cluster IV IR77734-93-2-3-2 is very distinct and less similar to others cultivars. The intra cluster distance among various clusters exhibited maximum intra cluster distance for cluster V (777.66) and lowest intra cluster distance was recorded for cluster IV (279.74). The maximum intra cluster distance was because of wide genetic diversity among its genotypes. The chance of developing good segregates by crossing the genotypes of the same cluster showing low value for intra cluster distance are very low. Therefore, it would be logical to attempt crosses between the genotypes of clusters separated by larger inter cluster distances. The little diversity and selection of parents within the cluster having higher mean for a particular character may also be useful for further developing high yielding rice varieties.

Table 2: List of SSR Primers and their sequences used for the analysis of Different cultivars of rice

S.No	Primer code	Make	Primer sequences	
			FORWARD	REVERSE
1	RM1013	Genei	5'GCTGCAATGTCTTTCACTGC3'	5'GGCTTTGGGGGAAATAGAAG3'
2	RM1019	Genei	5'GTTTGAACAGTAGGACTTGT3'	5'AGAACATCTCACACTTCTCT3'
3	RM1067	Genei	5'CGATGGAGAGAGAATGTCTAG3'	5'TAATACGCAAGGCAGAAGGG3'
4	RM1068	Genei	5'GCTGTACAGTGTCTTCGTTTC3'	5'TGCCTATTGCCTACTCACTC3'
5	RM1075	Genei	5'CCAGTTCAGTAGTTCACACAC3'	5'GTTGGGTTGCTGTGTTGTTC3'
6	RM1083	Genei	5'CCTTGATTGCAGCATCCG3'	5'TTGAGCCTTTTACGAGACGG3'
7	RM1090	Genei	5'GTTATAGCGCACCCTGGATG3'	5'GAACCGAAGGGACATGTGTG3'
8	RM1092	Genei	5'ACCCACCACCCTTTGACC3'	5'ATTTTGGTCTACGTGACGGC3'
9	RM1093	Genei	5'AGGTTGATGAACCCGATGAG3'	5'CTAGCTGCAGAACGGAGGAG3'
10	RM1095	Genei	5'CCCATTCAGTTGATCCTGTC3'	5'GCAAAAGCAAGGATGGAGAC3'

Table 3. Polymorphism Information Content (PIC) of SSR Loci across various cultivar analyzed in the present investigation

S. No.	Primer	Annealing Temperature (^{0}C)	Total No. of alleles	No. of polymorphic alleles	No. of monomorphic alleles	Diversity in value of PIC
1	RM1013	58	2	0	1	0.44
2	RM1019	54	3	0	1	0.59
3	RM1067	57	2	0	1	0.75
4	RM1068	59	1	0	1	0.097
5	RM1075	59	2	1	0	0.78
6	RM1083	56	2	0	1	0.69
7	RM1090	60	2	0	1	0.51
8	RM1092	57	2	0	1	0.097
9	RM1093	60	3	0	1	0.69
10	RM1095	58	5	2	0	0.87

CONCLUSSION

In this study molecular diversity of twenty rice cultivars was accessed though SSR markers. Diversity analysis in studies 20 Different cultivars evaluated on the basis of PIC (Polymorphism Information Content). All the rice cultivars showed high degree of genetic similarity. Genotype from the same geographical region fell into different clusters and vice-versa which, suggests that selections of parents for hybridization should be on genetic diversity rather than on the geographical areas.

Jaccard's coefficient of similarity derived from SSR data obtained by 20 Different rice cultivar and 10 primer

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	0.55	0.50	0.50	0.66	0.70	0.70	0.60	0.77	0.55	0.66	0.50	0.66	0.55	0.44	0.62	0.77	0.77	0.66	0.75
2		1	0.50	0.87	0.36	0.70	0.70	0.60	0.77	0.75	0.50	0.50	0.50	0.40	0.62	0.62	0.60	0.60	0.50	0.40
3			1	0.44	0.62	0.36	0.36	0.40	0.40	0.50	0.44	0.44	0.44	0.33	0.57	0.57	0.40	0.40	0.30	0.50
4				1	0.45	0.80	0.80	0.70	0.70	0.66	0.60	0.60	0.60	0.50	0.75	0.55	0.70	0.70	0.60	0.50
5					1	0.63	0.63	0.70	0.54	0.50	0.60	0.60	0.77	0.66	0.55	055	0.70	0.70	0.60	0.87
6						1	1.00	0.90	0.90	0.70	0.63	0.63	0.80	0.70	0.60	0.60	0.90	0.90	0.80	0.70
7							1	0.90	0.90	0.70	0.63	0.63	0.80	0.70	0.60	0.60	0.90	0.90	0.80	0.70
8								1	0.80	0.77	0.54	0.70	0.88	0.77	0.66	0.66	0.80	0.80	0.70	0.77
9									1	0.77	0.54	0.54	0.70	0.60	0.50	0.66	0.80	0.80	0.70	0.60
10										1	0.50	0.66	0.66	0.55	0.62	0.62	0.60	0.60	0.50	0.55
11											1	0.77	0.60	0.50	0.55	0.40	0.70	0.70	0.60	0.66
12												1	0.60	0.66	0.55	0.55	0.54	0.70	0.45	0.66
13													1	0.66	0.75	0.55	0.88	0.70	0.77	0.87
14														1	0.44	0.62	0.60	0.77	0.50	0.75
15															1	0.50	0.66	0.50	0.55	0.62
16																1	0.50	0.66	0.40	0.62
17																	1	0.80	0.88	0.77
18																		1	0.70	0.77
19																			1	0.66
20																				1

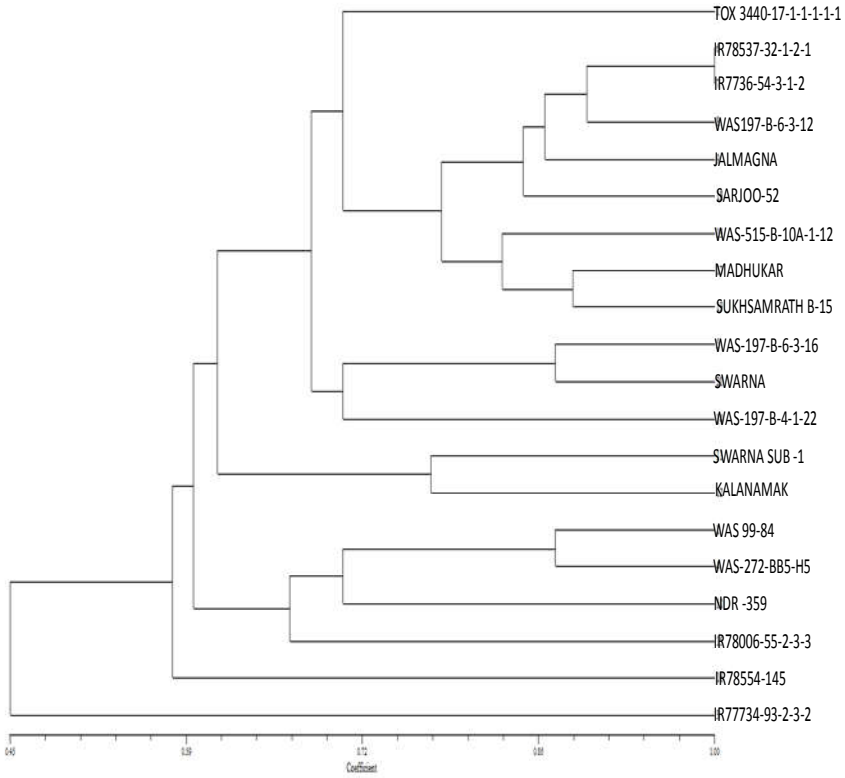

Fig. 1: **Dendrogram showing clustering of 20 rice varieties constructed using UPGMA based on Jaccard's similarity coefficient obtained from SSR analysis.**

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