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Analysis of Genetic Diversity using Molecular Markers among Some Elite Rice Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Microsatellites, also known as SSRs, are one of the most commonly utilised DNA marker types for a variety of applications, including genotypic differences, genetic relationships, QTLs, association mapping, genome mapping, and varietal identification. With the goal of assessing genetic diversity analysis of different varieties to measure the extent of genotypic differences, genetic relationship, and also to broaden the germplasm base of future rice breeding programmes, 40 rice genotypes were analysed for genetic variation using 20 SSR markers in the current study. These markers were discovered to produce polymorphic bands among the population, revealing the population's variability. Several alleles were found using microsatellite markers in this study, ranging from 2 to 5, with an average of 3.75 and 0.71 of average genetic diversity. The average PIC value for the current study was 0.62, indicating that the germplasm included in the study is diversified. This study exhibited that SSR primers such as RM1, RM25425, RM20224, RM18107, RM215, RM 248, and RM 535 with the genetic diversity greater than 0.70 and all of these are more polymorphic in nature in distinguishing the genotypes.

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1. INTRODUCTION

Rice (*Oryza sativa L.)* a "Global Grain" is one of the world's oldest cultivated and most significant staple food crops. The demand for rice in 2030 will be 30% higher than what is currently available [1] drought in some areas, salt and submergence in coastal areas, and rapid climate change will result in an increase in stressful situations. As a result of the alteration of established ecosystems, the development of novel types that are suited to such conditions is critical. The first step toward achieving this goal is to examine the current rice population. A comprehensive grasp of population structure and diversity provides insight into the likely evolutionary interaction between genotypes and the ancestry to which they belong. Individual genetic diversity demonstrates the occurrence of various alleles in the gene pool and, as a result, various genotypes within the population [2]. For the present study SSR microsatellite markers were used due to it's reasonably low cost, high estimations of polymorphism [3] which is highly utilizable for studying of phylogenetic relations among the genotypes belonging to three different ancestries like Indica, Japonica and Tropical japonica. In the absence of environmental influence, molecular markers can reveal significant differences across genotypes at the DNA level, giving a more direct, reliable, and efficient tool for germplasm characterization, conservation, and management. Rice has a lot of microsatellites, and over 2,500 of them have previously been produced as molecular markers [4,5]. Hence assessment of genetic diversity becomes important in establishing relationships among different cultivars [6,7]. Different qualities, such as salt tolerance/other abiotic stress, micronutrients, and so on, can be studied using genetic diversity analysis because they are influenced by a large number of QTLs that may share homology with genes responsible for other aspects.

With the goal of assessing genetic diversity analysis of different varieties to measure the extent of genotypic differences, genetic relationship, and also to broaden the germplasm base of future rice breeding programmes, 40 rice genotypes were analysed for genetic variation using SSR, markers in the current study.

2. MATERIALS AND METHODS

A total of 40 rice germplasms including two check varieties, one high yielding variety Shatabdi and another check was IR-64 were used for analysing genetic diversity using 20 molecular markers covering over 12 chromosomes (Table-1) were initially chosen based on the various earlier reported markers from Gramene web site [8].

The genomic DNA of all rice genotypes were isolated from 2-3 week seedlings following standard Cetyl trimethy lammonium bromide (CTAB) method [9]. The Polymerase Chain reaction was conducted in a reaction solution of 25ul containing template DNA, markers, dNTPs, reaction buffer, MgCl2 and Taq DNA polymerase. The PCR amplification was performed using an Eppendorf (Germany) thermo cycler, according to the cycle profile initial denaturation at 94˚C for 5 minutes, and then 36 cycles of 1 minute denaturation at 94˚C, 2minutes annealing at 55-57˚C and 2 minutes extension at 72˚C and 5 min at 72˚C for the final product extension.PCR products were subjected to electrophoresis in 2% Agarose gel in 1X TAE buffer at 120 volts, where the running time depended on the size of the PCR products from 20-35 minutes.DNA bands were visualised under UV light using the Gel Documentation Unit (UVP, UK).The size of amplified DNA was identified by comparing the distance migrated by the amplified fragment with respect to the molecular weight of a DNA marker whose size is already known (100 base pair (bp) DNA ladder, Fermentas). Presence and absence of allele and their specific size was scored for all the germplasms. The number of polymorphic alleles per locus, effective allele frequency, gene diversity and PIC values were calculated. A dendrogram was constructed to determine genetic diversity among the genotypes by applying un-weighted pair group method with arithmetic averages (UPGMA) clustering algorithm using IBM SPSS version 20.0 software.

Table 1. List of 20 Primers

Sl. no	Primer	Chr. no.	Forward Sequence	Reverse sequence	Repeat motif
1	RM1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	(GA)26
2	RM 535	2	ACTACATACACGGCCCTTGC	CTACGTGGACACCGTCACAC	(AG)11
3	RM 55	3	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	(GA)17
4	RM1305	4	ACCTGCATCGTATGTGTGTG	TCTGGGGTAAACACTGGGAG	(GA)19
5	RM 18107	5	CGTATGGACTTGCCTTGAGTCG	TCCAATCTGCCAAGCTTTACACC	(GA)33
6	RM 20224	6	AGTATGAAAGTCGGTGACGATGG	GAGATGTCACGTCTTCACTTAGGG	(CT)25
7	RM 542	7	TGAATCAAGCCCCTCACTAC	TCTGCAACGAGTAAGGCAGAG	(CT)22
8	RM 152	2	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	(GCT)8
9	RM257	9	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	(CT)24
10	RM 25425	7	CCAGCCCAAACAGCTCTTGC	GGGCACTGTTTGTCTTTCTGTGC	(GCT)8
11	RM 21	11	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	(GA)11
12	RM270	12	GGCCGTTGGTTCTAAAATC	GCGCAGTATCATCGGCGAG	(GA)13
13	RM489	3	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG	(ATA)8
14	RM 25	8	GGAAAGAATGATCTTTTCATGG	TACCATCAAAACCAATGTTC	(GA)18
15	RM 248	7	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	(CT)25
16	RM454	6	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG	(GCT)8
17	RM215	9	CAAAATGGAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	(CT)16
18	RM152	2	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC	(GGC)10
19	RM260	12	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	(CT)34
20	RM237	1	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	(CT)18

3. RESULTS AND DISCUSSION

These markers were discovered to produce polymorphic bands among the population, revealing the population's variety. Table 2 summarises SSR locus diversity results. SSR analysis revealed a total of 73 alleles, indicating that the population has a wide range of polymorphism. The number of alleles per microsatellite locus varied from 2 to 5 with an average of 3.75 alleles. The overall size of the amplified product varied from 75 bp (RM 21) to 400 bp (RM257) for this experiment. This wide range reflects a large difference in the number of repeats between different alleles. The level of polymorphism was evaluated by calculating PIC value of each of the 20 primers selected for the study. The PIC values indicate the highly informative nature of these microsatellites (Table no 3). Estimated PIC value ranged from 0.36 to 0.76.The mean PIC value was estimated at 0.62 which can be stated to be high according to Botstein et al. [10]. Genetic diversity (H_e) ranged from 0.59 to 0.85 and a highest value was observed for RM 1. Ferreira and Grattapaglia [11] concluded that microsatellite markers demonstrate high genetic diversity per locus because of their multi-allelism. The average PIC value (0.62)and Genetic diversity (H_e) (0.71) revealed that this study include a set of diverse genotypes.RM1, RM25425, RM20224, RM18107, RM215, RM 248,and RM 535 with the value of genetic diversity ≥ 0.70 showed more polymorphism.

3.1 Cluster Analysis

The dendrogram of 40 rice genotypes was constructed by the software NTSYS Pc Ver. 2.20 based on quantitative characters revealed four main clusters. It was clear from the clustering pattern that the genotypes belonging to different geographical origin were grouped together in the same cluster. In other words, genetic diversity is not related to geographic origin, where several varieties from the same zone may be distributed in different clusters.

Table 2. Characteristic of SSR markers used in the genetic assessment of rice genotypes

Sl. No	Primer name	Range	No. of Allele(A)	PIC Value	Genetic Diversity (H_e)
1	RM1	80-170	4	0.76	0.85
2	RM 535	80-150	4	0.69	0.75
3	RM 55	200-250	3	0.55	0.59
4	RM1305	200-360	3	0.64	0.69
5	RM 18107	150-250	4	0.72	0.75
6	RM 20224	100-250	5	0.70	0.82
7	RM 542	150-250	5	0.65	0.64
8	RM 152	108-163	3	0.54	0.68
9	RM257	260-400	4	0.59	0.65
10	RM 25425	130-310	4	0.72	0.84
11	RM 21	75-160	3	0.62	0.76
12	RM270	100-150	4	0.59	0.72
13	RM489	200-270	4	0.54	0.64
14	RM 25	130-148	3	0.69	0.71
15	RM 248	90-110	2	0.42	0.62
16	RM454	150-260	5	0.68	0.74
17	RM215	105-150	4	0.74	0.80
18	RM152	80-130	3	0.61	0.57
19	RM260	90-120	3	0.65	0.73
20	RM237	100-150	3	0.36	0.67

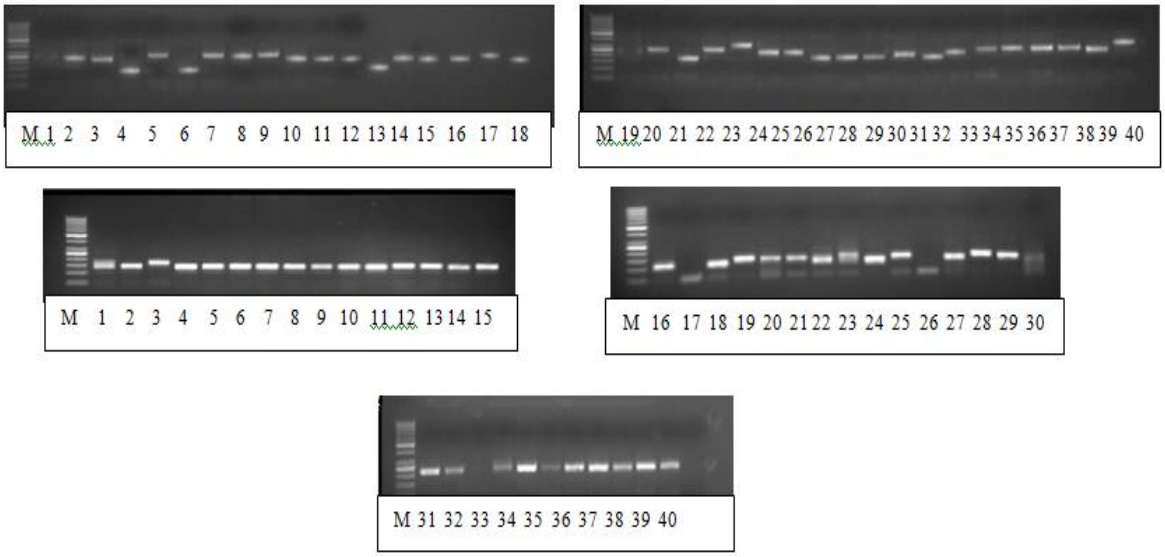

Fig. 1. Agarose gel showing SSR banding patterns with RM 215 and RM 1 primers with 40 rice genotypes

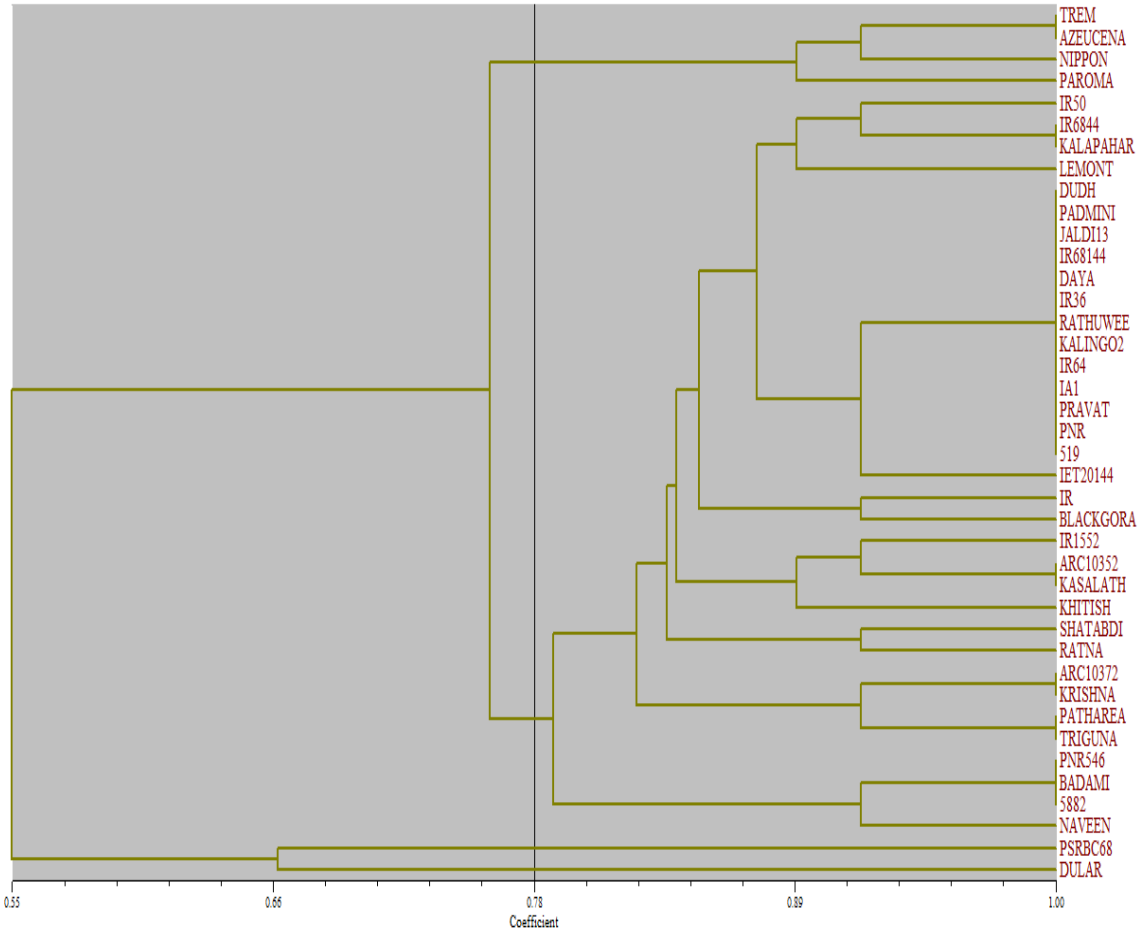

Fig. 2. Dendrogram based on SSR data for 40 rice genotypes

Table 3. Cluster Composition of 40 Rice Genotypes Based on SSR Data

Cluster	Sub Cluster	Name of genotypes
I		Tremebase, Nipponbare, Azeucena, Paroma-ahu
II	A	IR -50, IR-6844-120, Kalapahar, Lemont, Dudheswar, Padmini Jaldi-13, Daya, IR- 36, Rathuwee, Kalingo-2, IR-64, IA-1, Pravat, PNR-546, IET-20144, Blackgora, IR-1552, ARC-10352, Kasalath, Khitish, Satabdi, Ratna, ARC -10372, Krishna-hamsa, Patharea, Triguna, IR-68144-2B-2-2-2-1-12, PNR 519, Carolina-gold-sel, Bona-ahu
	B	Badami, Naveen, IR-5882-23-1-3-1
III		PSRBC-68
IV		Dular

4. CONCLUSION

The molecular markers are more reliable than morphometric markers in evaluating the genetic diversity precisely by distinguishing variations at DNA level and the pedigree records offer parentage information. Recently, the microsatellites or SSRs are amongst the most extensively used DNA marker types for various purposes such as diversity, genome mapping and varietal identification etc.

In SSR analysis a total of 73 alleles were observed which suggested a high range of polymorphism present in the population. The number of alleles per microsatellite locus varied from 2 to 5 with an average of 3.75 alleles. The overall size of the amplified product varied from 75 bp (RM 21) to 400 bp (RM257) for this experiment. The huge variation in the number of repeats between alleles reflects the large variability in the number of repeats. The level of polymorphism was evaluated by calculating PIC value of each of the 20 primers selected for the study. The PIC values indicate the highly informative nature of these microsatellites (Table no 3). Estimated PIC value ranged from 0.36 to 0.76.The mean PIC value was estimated at 0.62 which can be stated to be high according to Botstein et al., 1980. Genetic diversity (He) ranged from 0.59 to 0.85 and a highest value was observed for RM 1.

RM1, RM25425, RM20224, RM18107, RM215, RM 248, and RM 535 with the value of genetic diversity ≥ 0.70 showed more polymorphism. Microsatellite analysis could be used effectively for germplasm diversity analysis, according to the findings of this research. Furthermore, it clarifies the genetic constituent of the population before constructing any breeding strategies. This marker-based identification and differentiation could be helpful to preserve the integrity of high-quality rice varieties to benefit both farmers and consumers.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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