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MOLECULAR MARKER BASED GENETIC DIVERSITY ANALYSIS OF INTERNATIONAL RICE (*ORYZA SATIVA* .L) GERMPLASM

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ABSTRACT

The genetic variation and relationships among 32 rice genotypes of diverse origin were evaluated using 19 simple sequence repeat (SSR) primers. Among 19 SSRs primers, 17 showed 100% polymorphism. The number of alleles ranged from 2 to 10 with the average value of 4.37 per locus. Three primers generated a total of 5 rare alleles. Polymorphism information content (PIC) values of the primers ranged from 0.79 (RM 167) to 0.99 (RM 47 and RM 10) with an average of 0.84. Cluster analysis based on SSR banding pattern grouped the rice genotypes into 2 major clusters with additional sub-clusters. SSR analysis revealed that genotype AUS287 was distantly related to Kalonchi with Jaccard similarity coefficient of 0.64. SSR analysis resulted in a more definitive separation of clusters of genotypes, indicating a higher level of efficiency of SSR markers for the accurate determination of relationship between genotypes.

Key Words: Rice, SSR, Genetic Variation, Genotypes

INTRODUCTION

Rice, *Oryza sativa* L. (2n = 24) belonging to the family *Graminae* and subfamily *Oryzoidea* is the staple food for one third of the world's population and occupies almost one-fifth of the total land area covered under cereals. The world's rice production has doubled during last 25 years, largely due to the use of improved technology such as high yielding genotypes and better crop management practices. Further scope of crop improvement depends on the conserved use of genetic variability and diversity in plant breeding programmes and use of new biotechnological tools. As compared with other crop species, the genetic diversity in the world rice germplasm is quite large. Three subspecies *i.e.*, *indica*, *japonica* and *javanica* compose a large reservoir of rice germplasm including a variety of local landraces and genotypes (11, 12, 10). In addition, there are number of wild relatives that provide potentially valuable resources for the improvement of cultivated rice (11, 17).

Diversity based on phenological and morphological characters usually vary with environments and evaluation of these traits requires growing the plants to full maturity prior to identification. Protein or isozyme marker studies are also influenced by environment and reveal low polymorphism. Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Several molecular markers viz. RFLP, RAPD, SSR, AFLP and SNPS are presently available to assess the variability and diversity at molecular level. Microsatellite loci, also known as simple sequence repeats (SSR) are among the most commonly used molecular markers. Microsatellites are PCR-based markers that are efficient and cost-effective to use. Compared with other markers, they are abundant, codominant and interspersed throughout the genome. These markers can detect a significantly higher degree of polymorphism in rice (15, 16) which becomes ideal for studies on genetic diversity and intensive genetic mapping (6).

Tens of thousands of potential SSRs have been identified in rice, and over 25,000 have been developed as molecular markers (20, 13). These markers are currently being used to develop high density genetic maps, genotyping rice accessions, determine the genetic structure, optimize the assembly of core collections, and for marker-assisted breeding (13, 22 , 10). Keeping the above consideration in view studies were conducted to assess the genetic variability among 32 rice genotypes originated from different parts of the world.

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MATERIALS AND METHODS

Plant Material

Thirty two genotypes originated from different geographical locations of rice were used in the study viz. AUS287, Mapoi, Menyarhunei, Muyamba, Paiam, Palenema, Paliandeh, Pannel, Pansanko, Pataim, Payema, Pecalo, Tagba, Tatan, Goda Heenati, Kalonchi, Nang Quot, Tetep, Yeikoyon, Heen Sulai, Jaymis, Kaluwee, Madal, Maha Dikwee, Manchel Paragahakaleyan, USA Batapola Al, Koshihikari, Ausboro, Jaldungi, Nirboi, Vadai and Gota. Leaf samples of these genotypes were collected from the field experiments of Genetics Division, Indian Agriculture Research institute, New Delhi, India from 2011, Kharif season. The detail of the genotypes is presented in Table 1.

DNA extraction, PCR analysis and Gel electrophoresis

DNA was extracted from leaves according to modified CTAB method (15 a). Genomic DNA was purified using RNAse. Nineteen SSR markers synthesized by Bangalore Genie, Merck, India were used. The list of SSR primers and their sequences used for the analysis of thirty two rice genotypes is presented in Table 2. Polymerase chain reaction (PCR) was conducted in a reaction solution of 20 micro litre containing 3ul of templte DNA, 0.5ul of each primer, and 1ul of each dNTPs, 10 X reaction buffer and 0.5ul of taq DNA polymerase. The PCR amplification was performed using a thermal cycler (Astec programme temperature control system), according to the cycle profile: initial denaturation is at 95 ^{o}C for 1 minute and then 1 min annealing at the temperature 55 ^{o}C , and 2 min extension at 72 ^{o}C and 7 min at 72 ^{o}C for the final product extension. Amplification products were stored at -20 ^{0}C until further use. PCR-amplified products were subjected to electrophoresis in 4% polyacryl-amide gel in 1X TBE buffer. DNA bands were visualized under UV light using the gel documentation system.

Data Analysis

Only clear and unambiguous SSR markers were scored. All the genotypes were scored for the presence (score '1') and absence (score '0') of the SSR bands. Polymorphism information content (PIC) was calculated as described by (3) and modified by (1). The calculation was based on the number of alleles/locus. Genetic similarities were estimated from the matrix of binary data using Jaccard coefficient. The similarity coefficients were used for the cluster analysis of the rice genotypes utilizing the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) as described by (18). The analysis and dendrogram construction were performed using the NTSYS-pc version (19).

RESULTS AND DISCUSSION

A wide range of genetic diversity was observed among 32 rice genotypes evaluated using SSR markers. A total of 83 alleles were detected among different genotypes. The number of alleles per locus ranged from 2 to 10 with an average of 4.37 per locus. The larger the repeat numbers in the microsatellite DNA the larger the number of identified alleles. On per locus basis these values were comparable to those reported earlier by (2). These numbers were higher than the average of 2.9 alleles per locus for various classes of microsatellites reported by (21). High number of alleles was expected to be found when a large number of landraces from a wide range of geographical origins is included in the study (4).

A total 246 bands were scored from 19 primers of which no band was found to be monomorphic and all were 100% polymorphic with an average of 12.94 bands per primer. The number of polymorphic alleles ranged from 2 to 10 and 100% polymorphism was obtained with 17 out of 19 primers. Such result indicates that the genotypes under study are distantly related and genetically diverse. The PIC value, a reflection of allele diversity and frequency among the genotypes, also varied from one locus to another. The PIC value for the SSR loci ranged from 0.79 (RM 167) to 0.99 (RM 47) with mean value of 0.84. The higher the PIC value of a locus, the higher the number of alleles detected. Such observed PIC value was comparable to the PIC value of 0.78 as reported by (8). The PIC values were even high as compared to previous estimates for rice microsatellites as reported by (15, 22) as 0.68, 0.62, 0.68 respectively, demonstrating diverse nature of selected genotypes. Markers with PIC values of 0.5 or higher as demonstrated in this study are greatly informative for genetic studies and useful as a marker at a specific locus (7). The high PIC values in in current study can be due to co-dominant expression and multiple alleles

Table 1 : Origin and Characteristic features of genotypes included in the current investigation.

S. No.	Name of Genotype	Source/Origin	Characteristic Features
1.	AUS287	Bangladesh	Semi dwarf high yield inbred line
2.	Mapoi,	Sierra Leone	High yielding, long duration (160 days), medium height, bold grain.
3.	Menyarhunei	Sierra Leone	High yielding rainfed lowland rice variety
4.	Muyamba	Sierra Leone	Heat tolerant lowland genotype and
5.	Paiam	Sierra Leone	Rainfed lowland rice genotype and susceptible for BLB and Blast
6.	Palenema,	Sierra Leone	High yielding, long duration (180 days), medium height, long fine grain like basmati
7.	Paliandeh	Sierra Leone	High yielding, long duration (210 days), medium height, long bold grain
8.	Pannel	Sierra Leone	Alkaline tolerant with high yield , long duration (180 days) and bold grain
9.	Pansanko	Sierra Leone	Salt tolerant, long duration and bold grain
10.	Pataim	Sierra Leone	High yielding, long duration, medium height, long bold grain
11.	Payema	Sierra Leone	Salt tolerant, long duration and bold grain
12.	Pecalo	Sierra Leone	High yielding, long duration, medium height, long fine grain like basmati
13.	Tagba	Sierra Leone	Long duration, grown in flood plain area
14.	Tatan	Sierra Leone	Semi-deep water rice genotype with submergence tolerant
15.	Goda Heenati,	Sri Lanka	Semi deep water traditional landraces with submergence tolerant
16.	Kalonchi,	Bangladesh	Tolerance to flooding during germination and adapted to direct sowing cultivation in flooded paddy field in cool region.
17.	Nang Quot	Vietnam	Tolerance to flood conditions
18.	Tetep	Vietnam	The variety were flood-tolerant (due to their high elongation ability) and resistant to BLB and blast diseases.
19.	Yeikoyon	Liberia	Flood and submergence tolerance
20.	Heen Sulai	Sri Lanka	Traditional Rice genotype tolerance to floods, submergence and adverse soils.
21.	Jaymis,	Sri Lanka	Flood tolerant landrace with insect tolerant like Stem borer and BPH
22.	Kaluwee	Sri Lanka	Suitable for costal region and resistance to pests and tolerance to iron toxicity
23.	Madal	Sri Lanka	Flood tolerant landrace
24.	Maha Dikwee	Sri Lanka	flood tolerant suitable for flood prone areas and resistant for BPH insects
25.	Manchel Paragahakaleyan	Sri Lanka	Flood tolerant genotype with elongation ability
26.	USA Batapola Al	Sri lanka	Tall, flood and submergence tolerant
27.	Koshihikari	Japan	High yield improved genotype with good quality, grown in irrigated conditions
28.	Ausboro,	Bangladesh	Saline tolerant suitable for costal saline area
29.	Jalnidhi	India	Suitable for flood prone ecosystem due to kneeing and elongation ability
30.	Nirboi	Bangladesh	Indigenous landrace suitable for deep-water ecosystem
31.	Vadai	Bangladesh	Grown in deep water , submergence tolerant and photoperiod sensitive
32.	Gota	Bangladesh	Drought tolerant landrace, tall and photoperiod sensitive

(9). In current studies SSR primer RM 47, RM 10, RM 20,RM 247,RM 320, RM 561,RM 547, RM 566, RM 519, RMR, RM 544, RM222, RM206, RM264 generated higher levels of polymorphism and any of them can be used to differentiate between the 32 rice genotypes. The SSR dataset generated from these primer combinations are sufficient for robust estimates and that additional datasets do not change the relationships among the 32 rice genotypes.

The cluster analysis showed a significant genetic variation among the rice genotypes studied, with similarity coefficients ranging between 0.64 and 1.00. The dendrogram revealed that the genotypes derived from the genetically similar type clustered together.

Table 2 : List of SSR primers and their sequences used for the analysis of thirty two rice genotypes.

S. No.	Primer code	Primer sequences 5'-3'	
		Forward	Reverse
1.	RM 222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG
2.	RM 19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
3.	RM 561	GAGCTGTTTTGGACTACGGC	GAGTAGCTTTCTCCCACCCC
4.	RM 206	CCCATGCGTTTAACATTC T	CGTTCCATCGATCCGTATGG
5.	RM 566	ACCCAACTACGATCATCG	CTCCAGGAACACGCTCTTTC
6.	RM 519	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC
7.	RM 20	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
8.	RMR	ACGAGCTCTCGATCAGCCTA	TCGGTCTCCATGTCCCAC
9.	RM 346	CGAGAGAGCCCATAACTACG	ACAAGACGACGAGGAGGGAC
10.	RM 21	ACAGT AT TCCGTAGACGG	GCTCCATGAGGG TGGTA AG
11.	RM 167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC
12.	RM 10	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGTCC
13.	RM 536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC
14.	RM 264	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC
15.	RM 547	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATTCTCGTAGCG
16.	RM 247	TAGTGCCGATCGATGTA ACG	CATATGGTTTTGACAAAGCG
17.	RM 544	TGTGAGCCTGAGCAAT AACG	GAAGCGTGTGATATCGCATG
18.	RM 47	ACTCCACTCCACTCCCCA	GTCAGCAGGTCGGACGTC
19.	RM 320	CAACGTGATCGAGGAGAT C	GGATTTGCTTACCACAGCTC

Table 3 : Polymorphism Information Content (PIC) of SSR Loci among genotypes.

S. No.	Primer	Rare Alleles	Total No. of alleles	Molecular wt. range (bp)	Multiple Alleles	Highest Frequency Allele		No. of polymorphic alleles	No. of monomorphic alleles	% polymorphism	PIC value
						Size (bp)	Frequency				
1.	RM 222	0	5	200-240	0	200	40%	5	0	100.00	0.94
2.	RM 19	0	0	0	0	0	0%	0	0	0.00	0
3.	RM 561	0	6	110-180	0	180	31%	6	0	100.00	0.96
4.	RM 206	1	6	120-190	2	150	35%	6	0	100.00	0.91
5.	RM 566	0	5	250-300	0	290	28%	5	0	100.00	0.95
6.	RM 519	0	3	130-170	0	250	45%	3	0	100.00	0.95
7.	RM 20	0	4	220-300	0	290	40%	4	0	100.00	0.97
8.	RMR	0	8	200-290	0	240	29%	8	0	100.00	0.95
9.	RM 346	2	8	110-180	4	250	23%	8	0	100.00	0.89
10.	RM 21	0	0	0	0	0	0%	0	0	0.00	0
11.	RM 167	0	4	120-170	0	140,150	33.3%	4	0	100.00	0.79
12.	RM 10	0	2	160-180	0	180	75%	2	0	100.00	0.99
13.	RM 536	0	5	190-240	0	210	30.4%	5	0	100.00	0.88
14.	RM 264	2	10	160-200	0	180	20%	10	0	100.00	0.92
15.	RM 547	0	5	190-280	2	290	43%	5	0	100.00	0.96
16.	RM 247	0	4	140-180	0	160,170	33.3%	4	0	100.00	0.97
17.	RM 544	0	3	230-290	0	280,290	45.4%	3	0	100.00	0.95
18.	RM 47	0	3	180-210	0	190,210	33.3%	3	0	100.00	0.99
19.	RM 320	0	2	180-250	0	240	57%	2	0	100.00	0.97

Cluster analysis based on SSR markers divided rice genotypes into two major clusters and several sub-clusters according to genetic relatedness (Fig 1). Subsubcluster A contains four genotypes (Pansanko, Pataim, Payema and Tagba) which have same country of origin i.e. Sierra Leone and belongs to long duration maturity group. The sub subcluster B contains genotype Paiam and Pannel were found genetically similar and are long duration genotypes. High level of similarity was detected between closely related genotypes such as Paiam and Paliandeh (0.99), Paliandeh and Pannel (0.99). AUS287, on the other hand, was separated from the rest of the genotypes at a similarity coefficient of 0.75. The similarity coefficients of 'AUS287' with all the other genotypes ranged from 0.64 to 0.82, while 'Tagba' showed

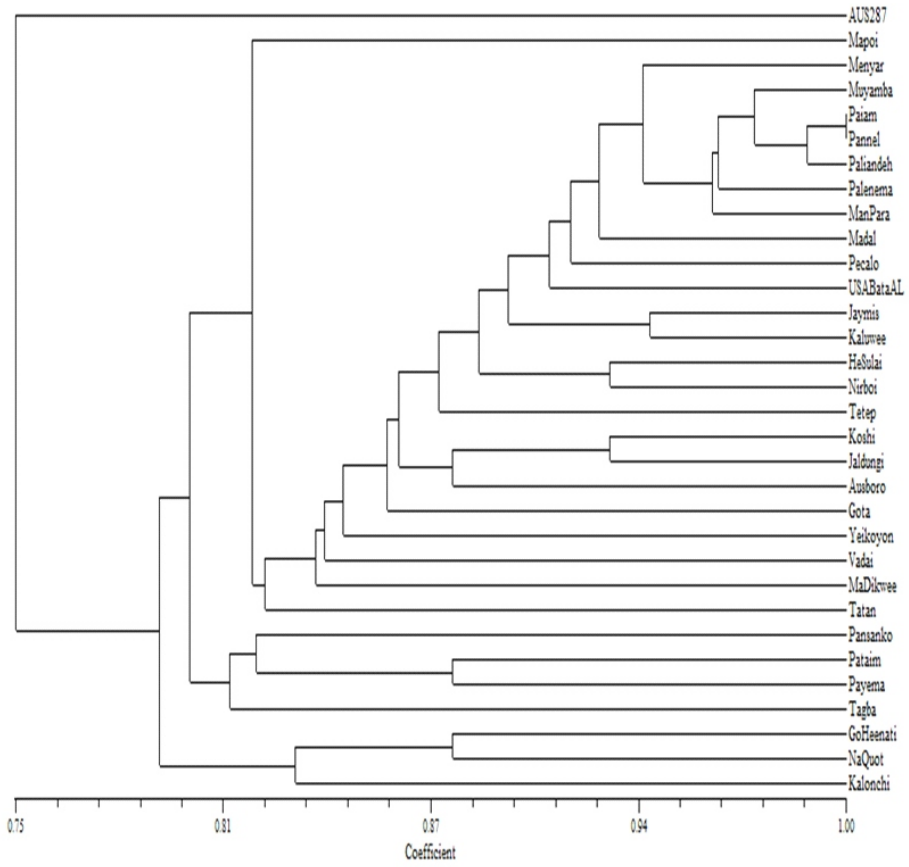

Figure 1: Dendrogram showing clustering of 32 genotypes of rice constructed by using UPGMA cluster analysis of genetic similarity based on SSR data.

similarity coefficients of 0.72 to 0.81 with all other genotypes . Similarly, the similarity coefficients of 'Pansanko' with other genotypes ranged from 0.72 to 0.86. Similar values of 0.77-0.98 were detected among 16 accessions of traditional, long-grain, scented Iranian rice and 7 genotypes from other countries (14). Similarity coefficients ranging from 0.36 to 0.96 were obtained among 45 accessions of AA-genome Oryza species from various locations suggesting a wider range of genetic variability (17). Amongst the analyzed genotypes, 'AUS287' and 'Tagba' showed strong dissimilarity to all other genotypes included in this study.

Based on current study large range of similarity values for related genotypes using microsatellites provides greater confidence for the assessment of genetic diversity and relationships. Results showed that microsatellite proved to be an efficient tool for diversity analysis. This analysis may help breeders to identify the parent's species from these genotypes for hybridization and optimum realization of heterosis. Moreover, the analysis of genetic diversity and relatedness between or within different species, populations and individuals will be of greater help in background selections during back cross breeding programs.

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