Indian Journal of Science and Technology, *Vol 12(28), DOI: 10.17485/ijst/2019/v12i28/142397, July 2019*

ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

DNA Fingerprinting and Diversity Analysis of Some Aus Rice Landraces

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Abstract

Objective: The allelic diversity and relationship among 48Aus rice landraces were determined through SSR markers. A total of 11 SSR markers were used to characterize all Aus rice genotypes. **Methods/Findings:** All of them were polymorphic showing different chromosome numbers. The number of alleles per locus varied from 3 alleles (RM234 and RM277) to 15 alleles (RM493). A total number of 72 alleles were detected with an average number of 8.91 alleles per locus. The polymorphic information content (PIC) values ranged from 0.19 (RM277) to 0.90 (RM493) with an average 0.70. The highest PIC value was obtained in RM493 (0.89) followed by RM526 (0.85) and The highest genetic dissimilarity (1.00) was found among the nineteen Aus genotypes combinations viz., Kalojira TAPL-68, Busmoti-sufoid, Hatisail, Sakharkhara, Khasa, Black, Ukinmodhu, Fulkari, Porbotjira, Straw THPL-554, Kalonunia, China IRRI, Mehedhan, Tulsimoni, Meny, Tilkapur, Nunia, Lalmota and Tapu. This material may be selected as potential parents for crop improvement for their distinctive characters. Whereas lowest genetic dissimilarity was found between Chini sagor-1 vs Malshira and Kalijir TAPL-74 vs Kalojira (0.18) followed by three other pairs (0.28). **Application:** The genetic distance-based results seen in the Unweighted Pair Group Method of Arithmetic means (UPGMA) revealed six genetic groups. These landraces will be useful for the selection of diverse parents, background selection during backcross breeding programs and assist in broadening germplasm-based rice breeding programs in the near future.

Keywords: DNA Fingerprinting, Genetic Diversity, Landraces, Microsatellites.

1. Introduction

Rice (*Oryza sativa* L.) landraces possess tremendous genetic variation. Evaluation of this terrific genetic diversity is very important for their rational use to diverse needs. It is not possible to meet up the striking demands of resource-restricted grower by the limited number of high-yielding and modern varieties[1]. Large number of high yielding and quality varieties are required to accomplish specific socio-economic and agricultural needs. Estimation of genetic parameters is thus thought to be useful in identifying the average genetic variation

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among the genotypes. The differences in allele frequencies among different populations and the differences in the level of polymorphism among populations will serve greatly in this purpose[2].

Understanding the population genetic structure will also be helpful in monitoring diversity loss over time and space, and also for devising a rational conservation plan for management of farmer landraces on-farm. Many factors (pattern of evolution, breeding methods, ecological and geographical factors, past bottleneck, human etc.) affect the extent and distribution of genetic diversity in a crop species. Maximum amount of this variation of a species may be observed within individual populations, or divided into various populations. Therefore, balanced use of germplasms and its conservation and management requires proper understanding of genetic diversity and its distribution in various individuals and species. Recently it has been identified that still there is a remarkable amount of unexploited genetic diversity exist in the rice primary gene pool. This can be used for increasing variability in local rice and for adaptation to different agro-ecological settings[3]. Wild species of *Oryza* may also serve as a valuable source of potential allele for biotic and a biotic stress resistance[4]. The Aus group of rice has a historically smaller geographical distribution and receive less attention than indica and japonica rice in breeding programs. But it has drought tolerance and early maturity potentially which traits could be useful in breeding program. As the number of rice cultivars increases, the ability to distinguish them on the basis of morphological and genetic traits becomes more difficult mostly due to genotype–environment interaction. Any developed or derived cultivar requires distinctness from its precursor for identity and protection. Both breeders and farmers tend to select among variations in their varieties and fields in order to maintain the purity or screen for a new type. Farmers select the best-performing plants from among the available varieties to compensate for micro-environmental variation. Therefore, without a good method for maintaining genetic purity of the varieties or cultivars, there is a danger of losing varietal identity. This is especially so where cultivation is mainly in small holdings with farmers of different skills, which increases the chances of genetic migration or genetic drift which is the variation in the relative frequency of different genotypes in a small population, where there is the chance of disappearance of particular genes as the varieties die or do not reproduce. Hence, the advent of plant variety protection lends added urgency to the search for solutions to the conservation of plant genetic diversity.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted at the Laboratory of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

2.2. Experimental Materials

In this study, forty-eight Aus varieties were selected as local land races. They were: Kalijira TAPL-68, Kalojira TAPL-74, Kalojira, Busmoti-sufoid, Bashmoti India, Dubsail, Dudsail, Chinisail, Hatisail, SagorDhana, Sadagura, Chini korai-2, Chini atob-2, Chini sagor-1, Chini sagor-2, Malshira, Jessobalam, Bash ful, Binna ful, Maloti, Khaskani, Bagunbitchi, Radunipagol, Sakharkhara, Khasa, Black, Ukinmodhu, Dhan-chikon, Fulkari , Khasamukpura, Porbotjira, Straw-THPL-554, Oval-TAPL-2990, Bowijali-2, Modhumadob, Tilokkochori, Bhatirchikon, Kalononia, China IRRI, Mehedhan, Tulsimoni, Kalogochi, Meny, Tilkapur, Nunia, Lalmota, Tapu and Kalomita.

2.3. DNA Extraction, Purification and Confirmation

Genomic DNA was extracted from 21-25 days old leaves using the mini preparation modified Cetyl Trimethyl Ammonium Bromide (CTAB) method. The quality of the isolated DNA in the protocol is sufficient for the PCR analysis[5]. DNA confirmation was done by using 0.8% agarose gel electrophoresis.

2.4. Documentation of the DNA Samples

After electrophoresis, the gel was taken out carefully from the gel chamber and transferred in a prepared ethidium bromide solution for staining. Staining was done for 20 minutes and then placed on the UV transilluminator in the dark chamber of the Image Documentation System. The UV light of the system was switched on. The image was visualized on the monitor and the photograph was saved in the Gel Doc computer.

2.5. Parental Survey and Primer Selection

Primers were evaluated based on intensity of bands, consistency within individual, presence of smearing and potentiality for population discrimination. In this experiment twelve random primers viz. RM493, RM526, RM5639, RM6659, RM334, RM314, RM234, RM342, RM171, RM536 and RM277 were used for parental surveys and selected. The sequences of the primers that are used for identification of salt tolerant rice germplasms are given at Table 1.

2.6. Allele Scoring

The size (in nucleotide base pairs) of the amplified band for each microsatellite marker was determined based on its migration relative to a molecular weight size marker (20bp DNA Ladder) with the help of Alpha Ease FC 4.0 software.

2.7. Analysis of SSR Data

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were determined using POWER MARKER version 3.23[6], genetic analysis software. Molecular weights for microsatellite products, in base-pairs, were estimated with Alpha Ease FC4.0 software. The individual fragments were assigned as alleles of the appropriate microsatellite loci. Polymorphism Information Content (PIC) values were described by[7] for self-pollinated species.

2.8. Nei's (1983) Genetic Distance

Nei's genetic distance and identity values were computed from frequencies of polymorphic markers to estimate genetic relationship between the studied 48 rice genotypes using the unweighted pair-group method with arithmetic means (UPGMA)[8].

3. Results and Discussion

Leaf sample collected from experimental field of Bangladesh Institute of Nuclear Agriculture (BINA) and analyzed by a highly repeatable PCR based fingerprinting assay known as Simple Sequence Repeat (SSR) or microsatellites markers.

3.1. Allelic and Loci Variation Within The Lines

The microsatellite enriched DNA fingerprint was carried out using the standard procedures. In this study 48 lines

Table 1. Sequence of selected primers with DNA band size

Primer	Product size (bp)	Forward primer	Reverse primer	Annealing temp. (°C)
RM493	211	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG	55
RM526	240	CCCAAGCAATACGTCCCTAG	ACCTGGTCATGACAAGGAGG	55
RM5639	123	GGAAGAACAGAGTTGCTCGG	GTGCCATTTATTTCCGTCCC	55
RM6659	101	GTTGTTGTTGTTGTGGACGG	CTGCCCTGAGTCCTATGAGG	55
RM334	182	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	55
RM314	118	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACACGC	55
RM234	156	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	55
RM342	141	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC	55
RM171	180	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	55
RM536	243	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC	55
RM277	124	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	55

of rice were analyzed using 11 primers (RM493, RM526, RM5639, RM6659, RM334, RM314, RM234, RM342, RM171, RM536 and RM277). Amplified microsatellite loci were analyzed for polymorphism study. The result revealed that all the primers detected polymorphism among the rice lines analyzed. The microsatellite loci showed polymorphism for 3 to 15 alleles per locus with a mean of 8.91 alleles per locus in the present study.

3.2. Size of Alleles

Highest amplicon size was 311bp produced by RM536 and the lowest amplicon size was 89 bp produced by RM277. The band size ranges of SSR markers found for RM 536 (237-311) and RM 493 (205-255) gave the range difference of 74 and 50 respectively (Table 2). Similar band sizes were noticed by[9, 10].

3.3. Number of Alleles Per Locus

Using 11 SSR markers, a total of 72 alleles were detected among the 48 rice lines. The average number of allele per locus was 8.91, with a range of 3 (RM234, RM277) to 15 (RM493) (Table 3) show that the number of alleles per locus ranged from 5 alleles (RM 275) to 15 alleles (RM 180), with an average of 9.7 alleles across the 30 loci obtained.

Table 2. Data on number of alleles, repeat motifs, allele range, number of rare alleles, number of null alleles and Polymorphism Information Content (PIC) found among 48 rice genotypes for 11 microsatellite (SSR) markers

Markers	No. of Alleles	Repeat Motif	Allele Ranges	Rare Alleles	Null Alleles	PIC
RM493	15	(CTT)9	205-255	6	0	0.8911
RM526	10	(CT)21	147-170	3	0	0.8529
RM5639	9	(AAG)13	126-150	5	0	0.7328
RM6659	12	(GTT)14	152-175	7	0	0.7996
RM334	11	(CTT)20	249-270	5	1	0.8392
RM314	11	(GT)8(CG)3(GT)5	190-217	5	1	0.8265
RM234	3	(CT)25	160-195	0	1	0.2944
RM342	11	(CAT)12	202-218	5	1	0.8361
RM171	8	(GATG)5	245-266	1	1	0.8154
RM536	5	(CT)16	237-311	2	1	0.3948
RM277	3	(GA)11	89-117	1	1	0.1783
Total	**72**	-	-	**40**	**7**	**7.4611**
Mean	**8.91**	-	-	**3.64**	**0.64**	**0.68**

Table 3. Data on sample size, high frequency alleles, availability, gene diversity and heterozygosity found among 48 rice genotypes for 11 microsatellite (SSR) markers

Markers	Sample Size	High Frequency Allele		Availability	Gene Diversity	Heterozygosity
		Size (bp)	Frequency (%)			
RM493	48	208	18.75	1.0000	0.8993	0.0000
RM526	48	228	18.75	1.0000	0.8672	0.0000
RM5639	48	118	37.50	1.0000	0.7648	0.0000
RM6659	48	128	33.33	1.0000	0.8186	0.0000
RM334	48	180	22.92	1.0000	0.8550	0.0000
RM314	48	113	29.17	1.0000	0.8429	0.0000
RM234	48	156	81.25	1.0000	0.3203	0.0000
RM342	48	138	25	1.0000	0.8524	0.0000
RM171	48	288	22.92	1.0000	0.8359	0.0000
RM536	48	248	75	1.0000	0.4175	0.0000
RM277	48	117	89.58	1.0000	0.1901	0.0000
Mean	**48**	**174.73**	**41.29**	**1.0000**	**0.6967**	**0.0000**

3.4. Rare Alleles

An allele observed in less than 5% of the 48 accessions was considered to be rare. Rare alleles were observed at all of the SSR loci with an average of 3.63 rare alleles per locus and a total of 40 across all the loci. In general, markers detecting a greater number of alleles per locus detected rarer alleles. Marker RM 6659 detected 15 alleles with the highest of 7 rare alleles.

3.5. Null Alleles

Totally absent of allele indicates null allele. In this experiment the average value of null allele is 0.64. All markers except RM 493, RM 526, RM 5639 and RM 6659 have one (1) null allele each.

3.6. Polymorphism Information Content (PIC)

PIC value of each marker can be evaluated on the basis of its alleles. According to the measure of the informative nature of microsatellites, the PIC values ranged from a low of 0.08 (RM277) to a high of 0.90 (RM209) and the average was 0.69. PIC values also showed a significant, positive correlation with the number of alleles and allele size range for microsatellites evaluated in this study. The observed PIC values are similar to previous estimates of microsatellite analysis in rice of 0.34–0.88 and 0.65–0.91.

3.7. Major Allele

Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. The size of the different major alleles at different loci ranges from 113bp (RM314) to 288bp (RM171). On average, 41.29% of the 48 rice lines shared a common major allele ranging from 29.17% (RM314) to 75% (RM171) common allele at each locus. On an average 42.13% of the 12 landraces shared a common major allele at any given locus by[11].

3.8. Gene Diversity

The highest gene diversity (0.90) was observed in loci RM493 and the lowest gene diversity (0.19) was observed in loci RM277 with a mean diversity of 0.70. It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles which revealed higher gene diversity. The result is quite consistent with result of[12] where they found that sorghum landraces conserve the 80% of the diversity observed in the wild.

3.9. Nei's Genetic Distance Analysis

Genetic similarities were calculated from the data of coefficient[13]. The similarly matrix was used to determine the level of relatedness among the studied genotypes. Pair-wise estimates of similarity ranged from 0.18 to 1.00 and the average similarity among all 48.The lowest genetic distance (0.18) was observed in Chinisagor vs Malshira and Kalijira TAPL-74 vs Kalojira. The highest genetic distance of 1.00 was observed between a number of accession or variety pair. Some of them were Kalijira TAPL-68 vs Straw TAPL-554, Khasa vs Porbotjira and other 18 pairs.

3.10. Cluster Analysis

Unweighted Pair Group Method of Arithmetic means (UPGMA) method was used for cluster analysis to differentiate the studied lines into groups based on similarity coefficient. Six clusters were made at genetic similarity level of 0.18 -1.00. The UPGMA cluster analysis led to the grouping of the 48 genotypes in six major clusters at genetic similarity level of 0.43 to 0.58. This cluster tree analysis agreed with the allelic diversity observed among Basmati and Non-basmati long grain indica rice varieties using microsatellite markers[14]. DNA fingerprinting and phylogenic analysis of Indian aromatic high quality rice germplasms also showed similar trend[15-18].

4. Conclusion

The present study was conducted to determine the genetic relationship among the genotypes for breeding purposes and to make DNA fingerprinting and genetic relationship among these lines using SSR markers. Microsatellites are considered appropriate for variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently. A minimum number of three microsatellite markers were sufficient for rapid and unambiguous discrimination of olive varieties. In another study, as few as six, well-chosen SSRs were sufficient to discriminate between 48 related lines of rice. In the present experiment, larger number of local cultivars was discriminated at the same loci compared with the

modern high yielding rice varieties. It is due to the fact that local varieties possess more genetic variability.

Allele scoring denotes that a total of 72 alleles were detected with an average number of 8.91 alleles per locus. From SSR data analysis, it was found that PIC values ranged from 0.08 (RM277) to 0.89 (RM493) and the average value was 0.68. The frequency of the most common allele at each locus ranged from 29.17% (RM314) to 75% (RM171). The size of the different major alleles at different loci ranges from 113bp (RM314) to 288bp (RM171). The UPGMA cluster analysis of genetic similarity showed that all 48 rice germplasms could be easily distinguished based on the information generated by the 11 SSR markers. Six clusters were obtained with similarity coefficients of 0.48. The study also confirmed the value of microsatellite loci for genetic diversity studies of rice landraces found in earlier studies. The diversity and the unique features of the Bangladeshi rice-landrace collections examined in this study could be quite relevant to both domestic and global rice development.

5. Acknowledgements

The authors are very much grateful to Plant Breeding Division and Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh for their kind laboratory help through research work.

6. Conflict of Interest

The authors declare that they have no competing interests.

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