

Diversity analysis of some selected rice genotypes through SSR-based molecular markers

M. H. Shahriar¹, A. H. K. Robin^{1*}, S. N. Begum² and A. Hoque¹

¹Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh- 2202 and ²Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202, Bangladesh

*E-mail: gpb21bau@gmail.com

Abstract

An experiment was carried out to assess the genetic diversity of advanced rice (*Oryza sativa* L.) breeding lines using three SSR markers viz., RM147, RM167 and RM215. Thirty T-aman advanced breeding lines at F₉ generation along with 4 check varieties were assessed. All three primers showed polymorphism. A total of 29 alleles were detected among the rice genotypes with an average of 9.67 alleles per locus. Polymorphism information content (PIC) ranged from 0.47 to 0.88 with an average of 0.71. A dendrogram was constructed based on total microsatellite polymorphism and 34 genotypes were grouped into four major clusters at 0.36 similarity co-efficient differentiating the early maturing genotypes from the late maturing types. The information about the genetic diversity might be utilized in future breeding programs for developing rice varieties with much shorter growth duration. The results also suggested that microsatellite markers which are linked to genes or QTLs responsible for growth duration properties are suitable tools for marker assisted selection (MAS) to select the rice genotypes of shorter growth duration.

Keywords: Rice, SSR marker, Polymorphism, Genetic diversity, Advanced breeding lines

Introduction

Bangladesh is a predominantly rice-based agricultural country. Rice being the most dominant food crop of Bangladesh accounted for 75 percent of agricultural land use and 28 percent of GDP (Anonymous, 1998). Rice-based agriculture currently occupies about 90% space in a cropping pattern nearly in all rice growing regions. In fact, rice-rice-rice crop rotation has forcibly occupied cropping fields to feed hungry teeming millions of Bangladesh. To increase crop diversity and to bring change in rice based cropping patterns, especially designed breeding schemes are often needed. These include developing of short duration, salt-tolerance, drought tolerance, disease resistance, and high protein or mineral content rice varieties.

Molecular markers are the powerful tools to detect genetic variation and genetic relationship within and among species. DNA markers are unmasking new genes for the improvement of crop varieties (Causse *et al.*, 1994). Generally, microsatellite loci are known as simple sequence repeats (SSRs) and these are the most popularly employed molecular markers (Litt and Lutty, 1989). They are basically PCR dependent markers which are efficient, convenient, cost effective as well as abundant, co-dominant, highly reproducible. SSRs can effective even in trace amount and also can interspersed throughout the genome (Panaud *et al.*, 1996, Temnykh *et al.*, 2000). In organisms, specific characters are represented by specific sequences and those sequences are easily identified by SSRs (Levinson and Gutman, 1987). Charismatic polymorphism and its degree of presence are also detected by SSRs markers (Ni *et al.*, 2002). That's the reason, why microsatellite markers have been widely applied in rice genome studies as well as allelic diversity analysis (McCouch *et al.*, 1997).

Keeping this point in view, the present investigation was undertaken to assess the genetic diversity of 30 advanced rice lines along with the check varieties to screen out the short duration lines and to select diversified parents for having desirable genotypes in segregating generations in future.

Materials and Methods

The experiment was carried out at the Biotechnology Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA) during the period of March- May 2014.

Plant materials

Seeds of 30 advanced breeding lines of T. aman rice along with 4 check varieties were collected from the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh (Table 1). Seeds were germinated in a controlled room by incubating those at 30°C in petridishes.

Table 1. A list of the genotypes used in the experiment

SL.	Germplasms	Sources
R1- R30	GPB accession, BAU	GPB, BAU
R31	BINA dhan 7	BINA, Mymensingh
R32	BRRI dhan 39	BRRI, Gazipur
R33	BRRI dhan 49	BRRI, Gazipur
R34	BRRI dhan 57	BRRI, Gazipur

Note: GPB= Department of Genetics and Plant Breeding, BAU= Bangladesh Agricultural University, BRRI= Bangladesh Rice Research Institute, BINA= Bangladesh Institute of Nuclear Agriculture

Collection of leaf samples

Young, vigorously growing fresh leaf samples were collected from 25 days old seedling of each of the thirty four genotypes which were used as the source of genomic DNA. Initially healthy portion of the youngest leaves of the seedlings were cut apart with a pair of sterilized scissors and washed in distilled water and ethanol and dried on a fresh tissue paper to remove spore of microorganisms and any other source of foreign DNA. Collected leaf samples were then put into polythene bags and kept on ice in an ice box. Tag was maintained for each sample. After that, the polythene bags were wrapped by aluminium foil and stored at -80°C freezer.

Genomic DNA isolation

DNA was extracted from the leaves of each genotype using the Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method (Stewart and Via, 1993).

SSR Protocol

PCR amplification of SSR markers was undertaken using three primer pairs enlisted in Table 2. Each of PCR reaction tube contained 3.4 µL ddH₂O, 3 µL template DNA, 0.2 µL dNTPs, 1.2 µL MgCl₂, 0.2 µL Taq polymerase, and 0.5 µL each of forward and reverse primers. Amplification was executed using the following conditions: preheated at 94°C for 3 minutes, 40 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 55°C, 2 minutes elongation at 72°C and a final extension at 72°C for 7 minutes. The SSR amplification products were separated through a vertical polyacrylamide gel electrophoresis unit. DNA fragments were expressed using ethidium bromide staining procedure. The gels were stained for 20 minutes. After that gels were documented using gel documentation unit (UV-trans-illuminator).

Table 2. Polymorphic SSR primers used in the present study for diversity analysis

Locus	Forward Primer	Reverse Primer	AT (°C)	PS (bp)
RM147	TACGGCTTCGGCGGCTGATTCC	CCCCCGAATCCCATCGAAACCC	55	97
RM167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC	55-60	128
RM215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	55	148

Note: AT= Annealing temperature, PS= Product size, position and number of repeats as previously published (<http://www.gramene.org>)

SSR data analysis

The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers and 25 base pairs (bp) DNA ladder using AlfaView software. The number of alleles per locus, major allele frequency, gene diversity, PIC, Nei's genetic identity and genetic distance values were calculated using PowerMarker version 3.25. MEGA5.22 software was used to construct a UPGMA (Unweighted Pair Group Method with Arithmetic Averages) dendrogram showing the distance-based inter-relationship among the genotypes.

Results and Discussion

In this experiment, all of three SSR motifs were polymorphic and produced varying number of alleles with different size ranges (Fig. 1). A total of 29 alleles were identified at the loci of 3 primers across 34 rice genotypes (Table 3). The number of alleles per locus ranged from 7 to 13 per locus. The locus RM147 had the highest number of alleles (13) and RM167 contained the lowest number of alleles (7). The average value of alleles is 9.67 per locus. The overall size of amplified products ranged from 76 bp in locus RM147 to 152 bp in locus RM215. On an average, 38% of the 34 rice genotypes shared a common major allele ranging from 17% (RM167) to 70% (RM147) common allele at each locus. A moderate level of diversity was obtained which ranged from 0.48 to 0.89 with an average of 0.73 among those 3 loci.

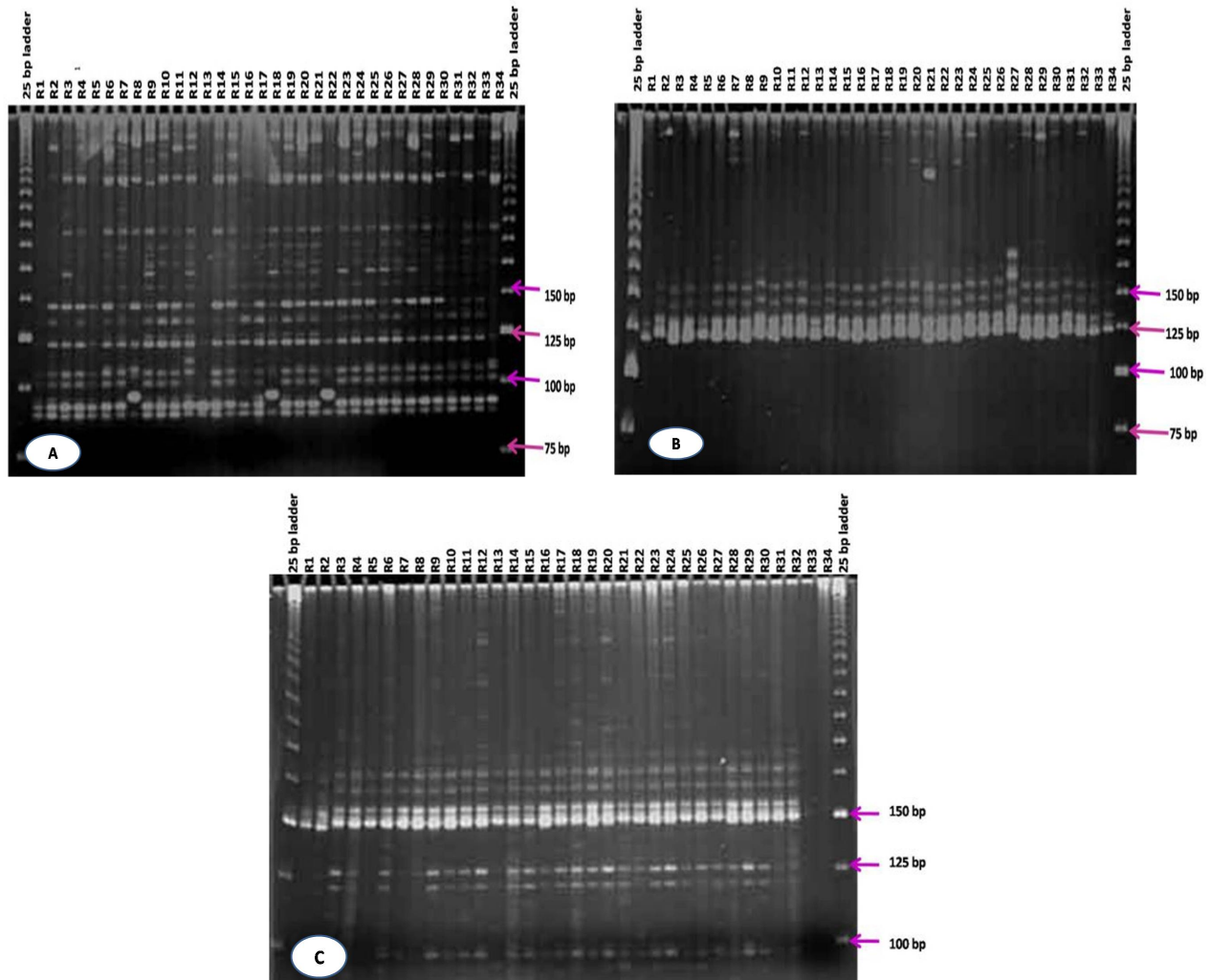


Fig. 1. SSR profile of 34 rice genotypes using primers RM147 (A), RM167 (B) and RM215 (C) respectively

Polymorphism information content (PIC) value is a reflection of allele diversity and frequency among the varieties. PIC value of each marker can be evaluated on the basis of its alleles. PIC varied significantly for all the studied SSR loci. In the present study, the level of polymorphism among the 34 genotypes was evaluated by calculating PIC values for each of the 3 SSR loci. The PIC values ranged from 0.47 (RM147) to 0.88 (RM167) with an average of 0.71 per locus (Table 3). These results revealed that markers RM167 would be the best in screening 30 rice genotypes followed by RM215 and RM147.

Table 3. Details of SSR markers showed polymorphism, indicating their location on rice chromosomes, number of alleles detected, allele size range, diversity and polymorphism information content (PIC)

Marker	Chromosome	No. of allele	Frequency	Size range (bp)	Gene diversity	PIC
RM147	10	7.00	0.70	85-92	0.48	0.47
RM167	11	13.00	0.17	120-135	0.89	0.88
RM215	9	9.00	0.26	140-148	0.81	0.79
Mean		9.67	0.38		0.73	0.71

Note: PIC= Polymorphism Information Content

Genetic similarities were calculated from the data of Nei's coefficient. The similarity matrix was used to determine the level of relatedness among the studied genotypes. Pair-wise estimates of similarity ranged from 0.00 to 1.00 and the average similarity among all 34 cultivars was 0.36.

UPGMA method was used for cluster analysis to differentiate the studied genotypes into groups based on similarity co-efficient. Four clusters were made at genetic similarity level of 0.3 -0.4 (Fig. 2). Cluster I, II, III and IV contained 8, 22, 2 and 2 genotypes respectively. The days to maturity of the genotypes of cluster I ranged from 112 to 119 days indicating all genotypes in this group were of comparatively shorter growth duration than the genotypes of other clusters (Fig. 2). This cluster includes the genotype R7 which required only 112.7 days for maturity (Shahriar *et al.*, 2014). Cluster II comprised of 21 advanced breeding lines and one check variety (BINA dhan 7). It is a cluster of mixture of short, intermediate and long duration genotypes with good yielding capability. A check variety (BRRI dhan 39) and a single advanced breeding line (R29) are included in cluster III. R29 had the highest filled grain capability. Cluster IV comprised of 2 check varieties *viz.*, BRRI dhan 49 and BRRI dhan 57.

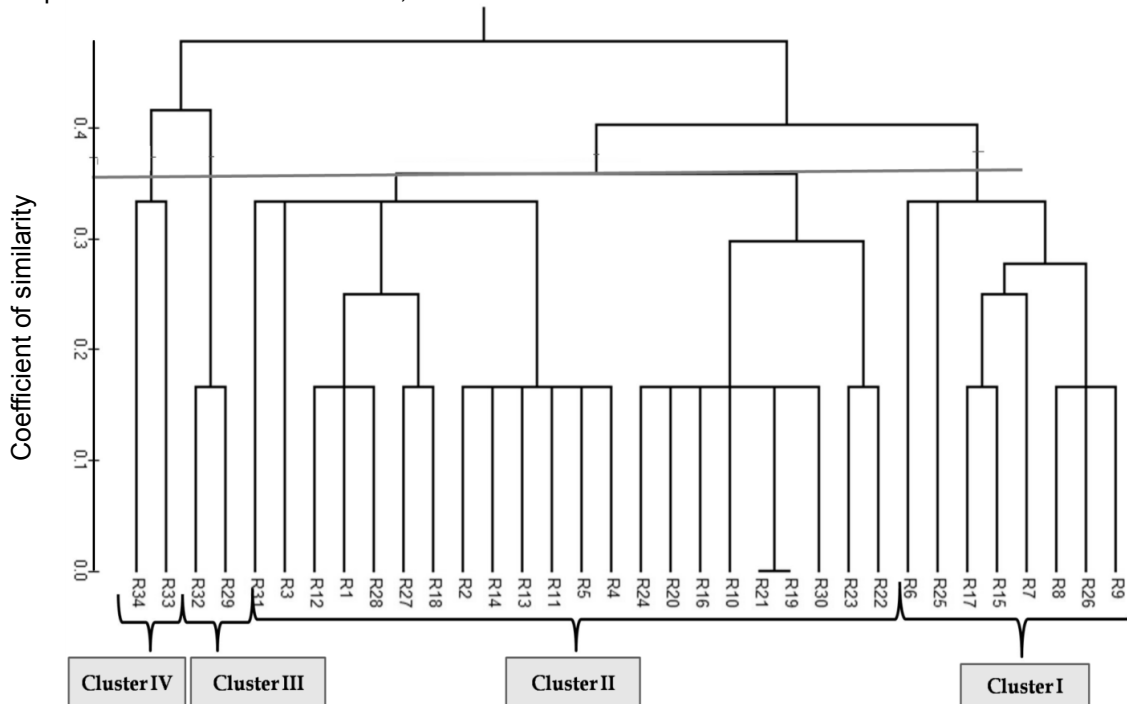


Fig. 2. UPGMA cluster dendrogram showing the genetic relationship among 34 rice genotypes

Conclusion

An SSR based screening to 34 rice genotypes using 3 markers selected a total of 29 alleles with an average of 9.67 alleles per locus. The highest PIC value was recorded for primer RM167 and that was the lowest for the primer RM147. Thirty four genotypes were grouped into 4 clusters where the genotypes of cluster 1 (8 genotypes including the earliest maturing genotype R7) were of shorter growth duration. SSR markers used in this study were convenient, neutral & co-dominant in nature.

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