Research Paper

Molecular approach to characterization of rice landraces of Eastern India with SSR markers

Biotechnology

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ABSTRACT

Rice, a model for cereal genomics, is the first crop genome to be sequenced. The present investigation aims at finding out the genetic diversity of seventeen elite rice cultivars of important agro-morphological traits for improved grain quality and higher grain yield using 10 SSR markers revealing high level of polymorphism among the landraces. A total of 22 scorable alleles were generated among the genotypes. The number of alleles produced by different primers ranged from two to three with an average of 2.2 alleles per locus. Cluster analysis categorized the cultivars into 6 clusters. The similarity coefficient ascertained through Jaccard's analysis revealed that genotype Gobindobhog and Jhingasail were more genetically diverged from the rest landraces. This study would benefit plant breeders, researchers providing useful information for selecting suitable parental genotypes for future crop improvement program and also in molecular breeding.

Introduction

Rice, the most important food grain crop, is cultivated on 154 million hectares world-wide. Rice germplasm in India is considered as a rich source of diversity due to the existence of high variability (Rao *et al*., 1979 and De *et al*., 1988). Genetic diversity has been studied by various means such as biometrical analysis, biochemical analysis and molecular markers. Crop improvement programmes at morphological and molecular level should necessarily aim at broadening the genetic base of the breeding stock. For the assessment of diversity pattern, the most commonly used markers are the phenotypic parameters and Simple Sequence Repeats (SSRs). Morphological and agronomic traits have long been the means of studying variability among populations and species (Gottileb, 1984; Bretting and Widrlechner, 1995). The determination of higher yield potential of the landraces, their local adaptation to the environment and of their capacity to escape from different biotic and abiotic stresses is documented by their expression of different agro-morphological features. Yet with the advent of DNA marker technology in the1980s offering a large number of genetic markers that were insensitive to environmental factors, the study of genetic variability and constitution was specific and facilitated to a greater extent. The inheritance pattern of the important agro-morphological traits can be studied with the generation of these markers (Peleman and Vander, 2003) to gain a specific view. Among different markers used, the most commonly used DNA marker types are microsatellites or SSR markers which are used in wide and broad aspects, like, in the field of analysis of genome diversity and mapping, characterization and varietal identification etc. In the present scenario of global research, SSR is the most popular and advantageous marker system in respect of being co-dominant, highly reproducible, highly polymorphic and abundant in animal, plant and microbe genomes and now in being utilized extensively in genetic mapping and gene tagging (Talukdar and Zhang, 2005), pedigree analysis (Rongwen *et al*., 1995) and genetic diversity studies (Struss and Plieske, 1998; Tenzer and Gessler, 1999).

The present study specifically evaluates the pattern and extent of genetic variability and diversity among seventeen elite rice landraces employing important agronomic traits with SSR markers. Thus, from this study, it could be concluded that these promising landraces can be used as a reservoir of beneficial gene pool in developing high yielding varieties in future breeding program.

Materials and Methods:

The present investigation used seventeen traditional lowland rice cultivars (Table 1) collected from the gene bank of Zonal Adaptive Research Station, Krishnagar, Nadia, West Bengal (23°24′N latitude and 88°31′E longitude with an altitude of 9.75 meters above mean sea level).

DNA extractions and PCR conditions

The DNA extraction from the seed of different cultivars was done following standard method (Murrey and Thompson, 1980). The genomic DNA was quantified and analyzed on gel electrophoresis. After drying overnight, DNA pillets were dissolved in 200µl of TE buffer (pH 8.0). DNA quantification was performed on 0.8% agarose gel and diluted with sterile distilled water to a concentration of 40ng for PCR analysis. The reaction mixture of PCR (20 µl) contained 12.8 µl of sterile distilled water, 2 µl 10× PCR assay buffer (50mM KCI, 10mM tris-cl, 1.5 mM $Mgcl_2$), 0.5 µl dNTP mix (0.2mM each of dATP, dTTP, dGTP and dCTP), 0.2 µl Taq DNA polymerase (1units), 0.5 µl primer each (0.5 µM) and 40ng template DNA. The following temperature conditions were followed to bring out the PCR amplification reaction in a thermocycler (Eppendorff): an initial temperature of 94°C for 5 min (first cycle), followed by 40 cycles of 94°C for 40 sec, 94°C for 40 sec, 55°C for 30 sec and 72°C for 45 sec. Final extension was done at 72°C for 10 min. Electrophoresis in 3% agarose gel in 0.5× TBE buffer (pH 8.0) at constant voltage at 80V for 3h. was done to resolve the amplified products. After staining and visualization of the DNA profiles on a UV transilluminator, photographs were taken by using gel Documentation System (Alpha Innotech, USA).

Diversity analysis by SSR markers

SSR Markers: Genotyping was done with a total of 10 previously developed SSR markers (McCouch *et al*.,2001). The Gramene website (www.gramene.org) provided the required Primer sequences. A score of clearly resolved unambiguous bands were taken visually for their presence or absence with each primer. The form of matrix with '1' and '0' delineated the obtained scores, indicating the presence and absence of bands in each variety respectively. The calculation of Polymorphic information content (PIC) values was done for each of the SSR loci using the formula developed by Nei (2002).

PIC$=1-\sum x^2_k\, n$

where x^2_k denotes the frequency of the K^{th} allele, and n the number of genotypes. The analysis of the data of microsatellite markers was done using NTSYS-pc statistical package, version 2.1 (Exeter software, Setauket, NY).

Results and Discussion

To determine the genetic diversity among seventeen elite rice cultivars (Table 1 & 2), a total of ten microsatellite markers were taken. Polymorphic patterns were displayed by all the ten SSR markers.

Table 1 list of rice landraces studied for the study of genetic diversity

Sl.No.	Genotypes	Source
1	FR 13A	ORISSA
2	KANAKCHUR	24 PARGANAS(S)
3	TULAIPANJI	WEST DINAJPUR
4	GOPALBHOG	MIDNAPUR(N)
5	KALONUNIA	WEST DINAJPUR
6	KALAMKATHI 147	BANKURA
7	SHIB CHATO	MIDNAPUR(N)
8	DOI MURI	MIDNAPUR(W)
9	KUMAR GORE	24 PARGANAS(S)
10	GOBINDOBHOG	BURDWAN
11	NC 678	MURSHIDABAD
12	SADAMOTA	24 PARGANAS(N)
13	GHEUS	24 PARGANAS
14	KANTARANGA	MIDNAPUR(W)
15	JHINGASAIL	BANKURA
16	SR 26B	ORRISA
17	GERUA MURI	MIDNAPUR(W)

Table 2 PIC and allele variation for SSR markers in selected promising landraces

S.No.	SSR	Chrom. no.	PIC Values	No. of alleles	Product size(bp)
1	RM-316	9	0.444	3	130
2	RM-208	2	0.722	2	180
3	RM-527	6	0.578	2	110
4	RM-247	12	0.472	2	160
5	RM-190	6	0.478	2	110
6	RM-21	11	0.556	3	143
7	RM-233	8	0.98	2	180
8	RM-342A	8	0.974	2	150
9	RM-1	1	0.5	2	100
10	AMS	2	0.99	2	280
	Total		6.694	22	
	Mean		0.669	2.2	

The PIC values of the polymorphic markers ranging from 0.444-0.99 with an average of 0.669 per primer do have a potential for discriminating the landraces taken under study (Table-2). Lower PIC value may come from closely related genotypes and higher PIC values might result from diverse genotypes. Juneja *et al*., (2006) reported earlier low PIC values for some other primers.

Among the primers in the present study, AMS is highly informative as it kept high PIC value (0.99). The markers presented an average PIC value of 0.669 denoting that SSR markers used in this study were highly informative because only PIC values higher than 0.5 indicate high polymorphism. In this present investigation, all the primers brought forth two to three alleles. Akagi *et al*. (1996) also observed such findings. Many studies have also cited significantly greater allelic diversity of microsatellite markers than other molecular markers (McCouch *et al*., 2001). A detection of total of 22 alleles occurred among the genotypes. The number of alleles per locus went from 2 to 3 with an average of 2.2 per locus.

SSR markers with multivariate nature contribute to the unambiguous advantage of discriminating genotypes more precisely. Allelic richness of six clusters (Table 3) for various sizes at a similar coefficient level of 0.75 could come to the fore by the cluster analysis. In order to choose the parents for successful hybridization, Jhingasail and Gobindabhog could be utilized for crossing with any of the other landraces from any other distantly related cluster. The application of more number of markers than used can give effective response to characterize the landraces for the present study which focussed the presence of diversity at genomic level among the genotypes studied.

Table 3 : Distribution of 17 elite landraces to different clusters

Sl. No.	Cluster	Number of genotypes	Name of the genotype(S)
1	I	3	Shib Chato, Gopalbhog, SR26B,
2	II	8	Kumar Gore, Kanta Ranga, Gerua Muri, NC678, Sadamota, Gheus, Tulaipanji, Kanakchur
3	III	1	Kalonunia
4	IV	2	Jhingasail, FR13A
5	V	2	Doi Muri, Kalamkathi147
6	VI	1	Gobindabhog

Morphological markers clustered all the genotypes at 10% of the genetic dissimilarity, while SSR markers clustered at 70% of genetic similarity. Different groups were obtained for the two types of the markers, which might be due to the fact that the evaluated traits for different markers were governed under different genetic background. Both morphological and agronomical traits were influenced by environmental factors (Morakinyo and Ajibade, 1998).

From the present study, it may be inferred that the rice cultivars taken for the study have many valuable traits which may be incorporated in future crop improvement programme The diversity analysis prompted the selection of 17 elite genotypes for their improvement of quality as well as desired agro-morphological traits. The data would help in selecting parents for crosses, in assigning cultivars to specific groups, and in precisely establishing relationship between landraces with the known cultivars. 10 SSR primers effectively established distinctiveness of 17 varieties by the amplification of different genotypes specific bands in theses varieties. Both the phenotypic parameters and molecular markers exhibited a high level of polymorphism, presenting genetic discrimination of the elite varieties. The grouping of genotypes into different clusters using molecular marker under this study has great utility in genetic diversity studies. Therefore, molecular markers like SSR could serve as a basic tool for the genetic diversity analysis and also for fingerprinting of closely related genotypes among the landraces

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