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Research Article

Assessment of Genetic Diversity across differentially adopted rice ecotypes

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Abstract:
Rice is an important staple food and a rich source of carbohydrates for 90% of South and Southeast Asians. Genetic diversity of naturally adopted upland lines along with few germplasm collections in Karnataka were studied based on 13 SSR loci on chromosome 1. A total of 24 individuals belonging to 3 differentially adopted groups [Upland (8), landraces (8) and cultivated varieties (8)] had a moderate to high level of genetic variability at groups level with number of alleles ranging from 5 to 23 (average 11.5) and PIC from 0.51 to 0.94 (average 0.79). The heterozygosity varied from 0.39 to as high as 0.70. Over all genetic diversity of 0.81 was revealed indicating a high level of genetic variation among these individuals. At the similarity coefficient of 7, all the lines were clearly grouped in 3 clusters. All cultivated types grouped in one cluster (cluster 1) except Rasi and CTH 1 which were included in cluster 2 and cluster 3 respectively. Cluster 1 contained 9 cultivated genotypes, where as cluster 2 contained 11 land races and cluster 3 had 4 upland lines. Out of 4 upland lines one line (AC-35341) intermixed, with C_1 and three lines (PS-229, JBT-37/4 and AC-35310) with C_3. The study of SSR markers on chromosome 1 facilitated the classification of these lines according to their adaptability. The information about the genetics of these populations will be for specific chromosomes and will be very useful for rice breeding programs, especially for gene mapping and ultimately for marker assisted selection programs.

Key Words: *Oryza sativa* L., SSR markers, Genetic Diversity, Cultivated, Landraces and Upland lines

Introduction

Rice (*Oryza sativa* L.) is an essential cereal crop grown in about one-third of the world's total cereal crop area and more than 90% of the rice is produced and consumed by South and Southeast Asians. Several studies showed that wild rice (*Oryza rufipogon Griff*.) Populations acquired significantly higher genetic diversity than the cultivated rice (*Oryza sativa. L*) (Khush, 1997, McCouch *et al*., 1997, Zho *et al.,* 2003). Domestication, artificial selection and intensive breeding of crops by human resulted in genetic bottleneck, which renders modern crop varieties more vulnerable to stresses. The vulnerability of a population to certain types of stresses can increase with reduction in genetic diversity. Low genetic variation within the species is an indirect display of inbreeding depression. SSR markers have been utilized in comparative studies to understand the wild relatives of rice, variety identification, genetic diversity, gene and quantitative trait locus analysis.

A species that has a large degree of genetic diversity among its population will have more variations from which to choose the fit alleles and it is essential for a species to evolve. Genetic variability and divergence present in the material is an important tool for any breeding programme. In the present study, an attempt is made to assess the molecular diversity at chromosome-1 using SSR markers among upland lines, land races and cultivars in India. Such information will have significance in providing the basis for selection of prebreeding material, conservation of resource material and also useful for rice crop improvement progarmme.

Material and Methods

Total of 24 lines comprising upland adopted lines (8), landraces (8) and cultivated varieties (8) were selected for the study (Table 1). Land races and upland rice accessions selected were adapted to upland conditions and some of the land races are presently being cultivated rarely in various parts of Karnataka. DNA was isolated from 20 days old rice leaf samples using cetyl trimethyl ammonium bromide (cTAB) method, purity of genomic DNA was assessed using Nanodrop quantifier (Biospec-nano, version 230V .name of manufacturer-Shimadzu biotech) by measuring the absorbance ratio of 260/280nm and also in running on 0.8 per cent

agarose. Dilutions were made in order to reduce the concentration to 20ng/µl. Totally 13 polymorphic rice SSR markers were selected from gramene database and custom synthesized from Bioserve Pvt, Limited, Hyderabad. Annealing temperatures of these were standardized using 15µl reaction mixture containing 2µl (20ng/µl) of DNA, 1.5 µl Taq buffer (1 X), 1.5 µl of dNTPs (3.0 mM), 0.3µl of $Mgcl_2$ (2mM), 1 µl each forward and reverse primers (5pMole.μL^{-1}), 0.3(1U) of Taq polymerase (Bangalore Invetrogen) and 7.4 µl of sterile water. The cycling conditions consisted of 94^0C for 5 min initial denaturation, followed by 30 cycles of 94^0C for 45 sec denaturation, specific temperature for each primer for 45sec annealing and 72 ^{0}C for 2 min polymerization, with a final extension of 8 min at 72 ^{0}C. The PCR products (15 µl) were subjected to fragment analysis with denaturing capillary electrophoresis using MCE-202 Microchip Electrophoresis System for DNA/RNA Analysis (MultiNA) Shimadzu microchip technology- Shimadzu, Japan. MultiNA is microchip based technology that is a new electrophoresis analysis platform which provides an alternative to agarose gel electrophoresis. The product size was visualized by comparing the sample peak with the ladder DNA-500 (25 bp DNA ladder) that has 19 peaks between 25 bp and 500 bp. Only the highest peak was selected as the PCR product.

The MultiNA scoring data of the SSR markers were used to calculate the number of alleles, frequency, heterozygosity (HE), Polymorphism Information Content (PIC) and genetic diversity of these markers using power marker software**.** The genetic similarity and distance was estimated by Nei's coefficient between pairs using neighbor joining algorithm and Dendrogram was constructed using NTYSYSpc 2.2.

Results and Discussion

Unlike the morphological and biochemical markers, molecular markers are not stressed by environmental factors and growth practices. Few reports pointed out microsatellites possessed hypervariability and resolving power among various marker systems (McCouch et al., 1997; Parida *et al*., 2009).

Development of rice cultivars suitable for semi-irrigated aerobic cultivations has been a major area of research. Since the technology saves 40 % of agricultural water, the adoption of rice is favored by many farmers. However rice suffers a significant economic yield penalty under water limited condition. It is important to estimate genetic diversity in existing cultivars in order to examine if the lack of genetic diversity is the limiting factor for crop improvement or genetic enhancement. Analysis of 24 rice cultivars using the 13 SSR primers on chromosome 1 displayed polymorphic amplicons and showed wider allelic variation among the selected loci. SSR loci on each of the chromosome 1 for all groups showed different level of genetic diversity. Number of alleles varied widely among the 13 loci and is summarized in Table 2. All of the 13 loci displayed polymorphism among 24 lines with a total of 150 alleles identified. The most variable locus (RM1387) had 23 alleles, and RM1074 produced only 5 alleles across 24 lines. The remaining loci had more than six alleles. Six to eleven alleles were resolved for 7 loci (RM140, RM237, RM165, RM212, RM297, RM10793, and RM8094) and 14-15 alleles were identified for four loci (RM493, RM302, RM3412 and RM3475). Genetic parameters in all the 13 SSR loci for 24 lines are given in Table III**.** Mean PIC, genetic diversity (GD) and heterozygosity among the 3 groups was 0.79, 0.81 and 0.37 respectively. Population mean of land races showed the lowest level of polymorphism (PIC= 0.59, GD= 0.64 and HE= 0.32), where as other two groups (upland lines and cultivated) showed the slightly greater level of PIC (0.65 & 0.70), GD (0.69 & 0.74) and heterozygosity (0.38 & 0.42) respectively (Table II)**.**

Dendrogram was constructed from the genetic similarity matrix using NTSYSpc software. Nei's neighbour joining algorithm was used for building the cluster tree. At a similarity coefficient of 7, the SSR markers were successful in revealing the presence of three broad groups among the genotypes. The phenogram indicated that the populations from the same group clustered together and were clearly separated into 3 major clusters (Figure 1). Cluster1 corresponded to the cultivated cultivars, whereas Cluster 2 corresponded to rice landraces and Cluster 3 corresponded to upland rice accessions. The similarity co-efficient for the three groups was almost on par ranging from 4.75 to 7.25 except for two genotypes (AC-35389, PS419) which stood away from other three groups with a distinct similarity coefficient of 10.00. Among the cultivated rice genotypes (Cluster 1) two cultivars namely Rasi and CTH-1 branched separately and stood among landraces indicating the similarity of these cultivars with the landraces. Among the rice landraces Nerguli stood between rice cultivars indicating the close relativity of Nerguli with Cluster1. Among the eight upland lines, two lines namely AC-35389 and PS-419 had the distinct similarity values of nearly 10.00 indicating that these upland lines are highly diverse lines compared to other rice genotypes (Figure 1). Apart from this 4 upland lines intermixed, one (AC-35341) with C_1 and three (PS-229, JBT-37/4 and AC-

35310) with C_3. This suggested that upland lines could have more similar genome with respect to chromosome 1 for 13 SSR loci's. As per Ni *et al* 2002 and Lapitan *et al* 2007, the information about the genetics of specific populations for a specific chromosome will be very useful for rice breeding programs, especially for gene mapping and ultimately for marker assisted selection programs. The data indicated that the upland rice accessions has sufficient genetic diversity and can be used to explore the possibility of developing semi-irrigated aerobic rice cultivar.

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Table 1. Rice genotypes evaluated for genetic diversity

Landraces	Cultivated	Upland
NERGULI	S9	PS419
HAMSA	RASI	AC35389
DBT	VANDANA	AC35006
DODDI	BPT36141	AC35310
PANKAJ	IET-15924	PS-229
HALUGIDDA	SOMSALI	JBT-37/4
KIRWANA	IR64	JBT36/169
DODDIGA	CTH1	AC35341

Table 2. Genetic parameters of 24 populations based on 13 microsatellite loci on chromosome 1.

	All	Land races	Upland	Cultivated
Allele frequency	0.32	0.5	0.42	0.39
Allele Number	11.5	4.8	4.9	5.9
Gene Diversity	0.81	0.64	0.69	0.73
Heterozygosity	0.37	0.32	0.38	0.41
PIC	0.79	0.59	0.65	0.7

Table 3. Data on the allele frequency (AF), alleles number (AN), gene diversity (GD), heterozygosity(HE) and polymorphism information content (PIC) of 13 microsatellite loci on chromosome 1 across 3 groups of populations.

	All groups					Land races					Upland					Cultivated				
SSR Locus	AF	AN	GD	HE	PIC	AF	AN	GD	HE	PIC	AF	AN	GD	HE	PIC	AF	AN	GD	HE	PIC
RM1074	0.61	5.00	0.56	0.04	0.51	0.86	2.00	0.24	0.00	0.21	0.63	3.00	0.53	0.00	0.47	0.56	3.00	0.54	0.13	0.45
RM10793	0.48	11.00	0.73	0.21	0.71	0.50	6.00	0.70	0.13	0.67	0.50	5.00	0.69	0.25	0.65	0.44	5.00	0.70	0.25	0.66
RM1387	0.14	23.00	0.94	0.94	0.94	0.20	9.00	0.88	1.00	0.87	0.19	12.00	0.90	1.00	0.89	0.30	6.00	0.80	0.80	0.77
RM140	0.31	6.00	0.77	0.00	0.73	0.50	4.00	0.67	0.00	0.62	0.60	3.00	0.56	0.00	0.50	0.60	3.00	0.56	0.00	0.50
RM165	0.36	9.00	0.76	0.91	0.72	0.50	3.00	0.56	1.00	0.46	0.44	5.00	0.71	0.88	0.67	0.29	6.00	0.80	0.86	0.77
RM212	0.33	9.00	0.82	0.00	0.80	0.80	2.00	0.32	0.00	0.27	0.43	3.00	0.65	0.00	0.58	0.33	5.00	0.78	0.00	0.74
RM237	0.36	8.00	0.81	0.00	0.79	0.50	3.00	0.63	0.00	0.55	0.40	3.00	0.64	0.00	0.56	0.60	3.00	0.56	0.00	0.50
RM297	0.28	9.00	0.84	0.39	0.82	0.25	6.00	0.80	0.38	0.78	0.29	5.00	0.79	0.43	0.75	0.44	5.00	0.73	0.38	0.69
RM302	0.26	15.00	0.88	0.09	0.87	0.57	4.00	0.61	0.00	0.57	0.25	7.00	0.82	0.13	0.80	0.19	8.00	0.87	0.13	0.85
RM3412	0.24	15.00	0.87	1.00	0.86	0.33	7.00	0.79	1.00	0.76	0.33	4.00	0.72	1.00	0.67	0.19	11.00	0.89	1.00	0.88
RM3475	0.34	15.00	0.82	0.86	0.80	0.31	9.00	0.84	0.63	0.82	0.43	4.00	0.62	1.00	0.55	0.29	8.00	0.83	1.00	0.81
RM493	0.26	14.00	0.86	0.38	0.85	0.57	4.00	0.61	0.00	0.57	0.50	6.00	0.69	0.29	0.66	0.36	9.00	0.82	0.86	0.80
RM8094	0.18	11.00	0.88	0.00	0.87	0.57	4.00	0.61	0.00	0.57	0.43	4.00	0.69	0.00	0.64	0.50	5.00	0.69	0.00	0.65
Mean	0.32	11.54	0.81	0.37	0.79	0.50	4.85	0.64	0.32	0.59	0.42	4.92	0.69	0.38	0.65	0.39	5.92	0.74	0.42	0.70
Mini	0.14	5.00	0.56	0.00	0.51	0.20	2.00	0.24	0.00	0.21	0.19	3.00	0.53	0.00	0.47	0.19	3.00	0.54	0.00	0.45
Max	0.61	23.00	0.94	1.00	0.94	0.86	9.00	0.88	1.00	0.87	0.63	12.00	0.90	1.00	0.89	0.60	11.00	0.89	1.00	0.88

Figure 1. Dendrogram of 24 rice lines derived from UPGMA cluster analysis using Nei's coefficient between pairs using neighbor joining algorithm based on 13 polymorphic SSR markers.