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Random amplified polymorphic DNA analysis of indigenous small and medium-grained scented rices (*Oryza sativa* L.) of Orissa

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Abstract

Scented rices are highly preferred due to their pleasant aroma and palatability. A large number of indigenous aromatic rice collections are awaiting systematic study for the assessment of genetic diversity. Random amplified polymorphic DNA technique was used for genetic diversity analysis among 24 rice cultivars consisting of 20 non-basmati scented and 4 non-scented rice varieties collected from Orissa. A total of 86 bands were amplified by 15 pre screened polymorphic decamer primers at an average rate of 5.73 bands/primer. The polymorphic loci recorded in the present study clearly discriminate all the accessions. The pair-wise genetic similarity coefficient varied from 0.52 between three pairs of rice genotypes viz., Samudrabali - Nariyaphula, Gayatri-Nadiarasa and IR64 - Gurmatia to 0.94 between Gurmatia and Samudrabali. The UPGMA cluster analysis revealed considerable variability among the population of 24 cultivars and discriminated all the non-scented rices from scented rice varieties. All the 24 rice genotypes were classified into three clusters and separated the non-scented rice varieties from rest of the scented rices. The group consisting of Nadiarasa and Nariyaphula with pair wise similarity coefficient 0.75 was separated from rest of the varieties at 60% similarity level. No duplicate entry was detected among the 24 rice cultivars included in the present study.

Key words: Scented rice, RAPD, cultivar identification, genetic diversity, *Oryza sativa*

Introduction

Scented rices are nature's gift to the Indian sub-continent and human kind at large. Compared to other class of rices, scented rices are highly demanded and get better premium in global market due to its pleasant aroma, superfine long-slender grains with delicate curvature, remarkable linear elongation and excellent flaky soft texture on cooking [1]. With the growing demand for these rices in international market, high emphasis has been placed for the improvement of basmati types. The long-grained basmati rices are generally exported and have assured markets, while the small and medium-grained scented rices being regarded as a separate class of non-basmati scented rice have limited popularity [2]. Orissa is endowed with a large number of such non-basmati rice varieties. These indigenous rice varieties blessed with some of the out standing qualities like very strong aroma, kernel elongation after cooking, fluffiness and taste and are quite popular in their native areas of cultivation and possesses a domestic market value. High degree of genetic variability for several agro-morphological and quality traits has been exhibited among this group of scented rice germplasm. Many of these varieties often possess a number of desirable yield traits like longer panicle, higher panicle number, larger and active sink with improved fertility and better test weight indicating their utility as potential donor for improvement of quality and yield parameters [3, 4]. The entire range of quality as well as yield traits are neither exploited nor fully utilized in breeding programs for the improvement of cultivars. Since the knowledge of genetic diversity in crop plants is the key to success in breeding programs, un-ambiguous, reliable, fast and cost effective determination of genetic diversity in plant verities is essential for the varietal identification, classification, proper purity maintenance and conservation.

Genetic diversity studies on rice using traditional morphological and biochemical markers are common and routinely used [5, 6]. Supplementing to above parameters PCR-based molecular markers are considered more suitable for analysis of genetic diversity and varietal identification since there is little effect of stage of development, environment or management practices.

A wide range of research works have been done on rice applying molecular markers *viz*., Restriction Fragment Length Polymorphism [7], Random Amplified Polymorphic DNA [8, 9], Simple Sequence Repeats [10-12], Amplified Fragment Length Polymorphism [13-15] and Inter Simple Sequence Repeats [16, 17]. All these molecular techniques have the common objective of assessing the relative diversity within and among the species and also to select the diverse accessions for breeding purposes.

In most of the studies basmati type of scented rices are clustered into a group distinct from the *indica* and *japonica* rice varieties [12, 14, 17]. The various molecular markers used for the plant genome analysis have their own limitations, necessitating careful evaluation of diversity analysis. Since genetic differentiations are often correlated with geographic isolation, it may be appropriate to analyze the germplasm that represent a wide range of geographic region in order to estimate the genetic diversity within the breeding stock. A little work has been done on the study of genetic variability among small and medium grained non-basmati scented rice germplasm of Orissa at molecular level. Among the various molecular marker techniques available RAPD analysis is considered to be technically simple and rapid DNA finger printing method. In the present study RAPD was used to evaluate the genetic diversity and to develop DNA profiles of indigenous non-basmati type rice germplasm representing diverse geographic locations of Orissa.

Materials and methods

Plant material

A total of 20 indigenous non-basmati scented rice genotypes, representing a wide range of geographic locations of Orissa and four non-scented high yielding rice varieties were selected for the present study. Wide range of variabilities was measured for seed morphology (size, shape, lemma and palea colour) in the germplasm based on the geographical areas of their cultivation. The genotypes were collected from NBPGR, New Delhi and Central Rice Research Institute, Cuttack. The details of the cultivars are given in Table 1.

Table 1. Details of rice genotypes used in the study

S.No.	Genotypes	Accession number	Areas of cultivation
1.	Basuabhog	IC 283118	Kandhamal
2.	Gurmatia	IC 283119	Puri
3.	Laxmisiali	IC 259013	Dhenkal
4.	Nariyaphula	IC 258852	Bhadrak
5.	Dubraj	IC 373222	Nawarangpur
6.	Maguraphula	IC 373145	Bargarh
7.	Samaleibhoga	IC 373134	Jharsuguda
8.	Maharaji	IC 373202	Kalahandi
9.	Gangabali	IC 305935	Jaypore
10.	Lectimachi	IC 321769	Nawarangpur
11.	Kainchaphula	IC 280540	Nayagarh
12.	Kalajira	IC 259032	Dhenkanal
13.	Mashiphula	IC 257258	Ganjam
14.	Gopalbhoga	IC 257566	Khurda
15.	Dhusara	IC 257670	Jagatsinghpur
16.	Haldilgundi	IC 283003	Cuttack
17.	Tulasibasa	IC 280552	Nayagarh
18.	Lalat	HYV	Orrisa
19.	Neela	HYV	Orrisa
20.	IR-64	HYV	Orrisa
21.	Gayatri	HYV	Orrisa
22.	Nadiarasa	IC 257290	Ganjam
23.	Karpurakanti	IC 321832	Kandhamal
24.	Samudrabali	IC 321674	Kandhamal

DNA isolation

Genomic DNA from each rice cultivar was isolated from etiolated leaf samples following (N-Cetyl) N, N, N - Trimethyl Ammonium Bromide (CTAB) method [18]. The DNA was spooled out, washed twice with 70% ethanol and dissolved in T.E. (10 mM Tris, 0.1 mM EDTA, pH 8.0) containing 25 µg/ml RNase-A, incubated at 37°C for 30 min and extracted with chloroform : isoamyl alcohol (24 : 1 v/v). DNA was re-precipitated and dissolved in T.E. buffer. The quality and quantity of DNA was checked by 1% agarose gel electrophoresis using standard containing 100 ng/µl genomic DNA. The isolated DNA samples for PCR were diluted in TE (9:1) to a working concentration of approximately 50 ng/µl and stored at 4°C until PCR amplification.

Primers

Fifteen pre-screened polymorphic decameric random primers synthesized from Bangalore Genei Pvt. Ltd., India, were used for the present investigation. The primer sequences and their GC contents are given in Table 2.

DNA amplification and gel electrophoresis

The amplification of target DNA was done according to

Table 2. List of random primers and their sequences

S.No.	Primer code	Sequences (5' to 3')	GC content (%)
1.	57ss	GGGTAACGCC	70
2.	63ST	GTGTGCCCCA	70
3.	62ss	GTCCCGACGA	70
4.	58ST	AATGCCCCAG	60
5.	65ss	CCACACTACC	60
6.	60ss	GGC TGCAGAA	60
7.	67ss	AGGCGGGAAC	70
8.	66ST	GCAGACTGAG	60
9.	68ss	AGGCGGGTAC	70
10.	69ST	CGGAGAGCGA	70
11.	E98932	GTGACGTAGG	60
12.	E98934	GTGATCGCAG	60
13.	E98933	GGGTAACGCC	70
14.	E98936	CAGCACCCAC	70
15.	E98937	TTCCGAACCC	60

the method of Williams *et al.* [19] in a peltier-cooler thermal cycler (PTC 200, MJ Research Inc., Water town, Massachusetts, USA) using 25 µl reaction mixture containing 1 X reaction buffer (10 mM Tris HCl, pH 8.3 and 50 mM KCl), 3.0 mM $MgCl_2$, 1.5U of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., India), 200 µm each of dATP, dTTP, dGTP and dCTP. 40ng of 10 nucleotide primer (Bangalore Genei Pvt. Ltd., India) and approximately 50 ng of genomic DNA template. The PCR amplification conditions were as follows: an initial denaturation at 94°C for 5 min and 35 cycles at 1 min denaturing at 94°C, 1 min annealing at 40°C and 2min polymerization at 72°C and 5 min final extension at 72°C. PCR products were mixed with 2.5 µl of gel loading dye and electrophoresed on 1.5% agarose gel in 0.5X TBE buffer at 80 volts. The gels were stained in Ethidium Bromide (0.5 µg/ml) and the gel image was recorded using the gel documentation system (Bio-RAD).

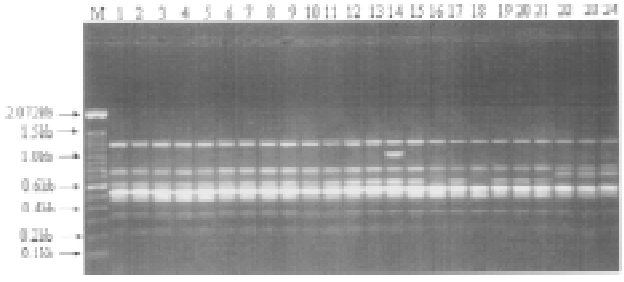

Fig. 1. Amplification of 24 rice genotypes using primer 62SS as per the genotype in sequence code in lane 1-24

Data analysis

RAPD banding patterns were scored as present (1) or absent (0) for each primer-genotype combination. Jaccard's similarity coefficient values for each pair wise comparison between cultivars were calculated and a similarity coefficient matrix was constructed. This matrix is subjected to Unweighted Pair Group Method for Arithmetic Average analysis (UPGMA) to generate a dendrogram. The similarity coefficient analysis and the dendrogram construction were carried out by using NTSYS-pc version 2.1 Software [20].

Results and discussion

RAPD analysis

The RAPD pattern of genomic DNA of 24 rice cultivars were analysed with respect to the distribution of the fragments, informativeness of the markers and polymorphism for the assessment of genetic diversity present among the genotypes. All the primers were found to produce polymorphic amplification products. The extent of polymorphism with a primer varied between 30% (Primer E98937) to 100% (four primers *viz*., 58ST, 60SS, 66ST and E98934). The reason for existence of higher percentage of polymorphism may be because of variations in the geographical distribution and seed morphology of the cultivars included in the study. A total of 86 bands were amplified using 15 decameric primers with an average of 5.73 bands per primer (Table 3). Of these, 65 bands were polymorphic showing 75.6% polymorphism. The number of bands amplified by the primers ranged from 2 (65ss) to 10 (E98937). A representative DNA profile for all the 24 cultivars using primer 62ss is shown in Fig. 1. Thus, all the cultivars could be distinguished from one another by the use of one or more primers. Primer 62ss and primer 66ST produced cultivar specific bands in the varieties Gopalbhog and IR-64, respectively. Thus, these two primers can be used for identification of specific cultivar.

Genetic relationship among cultivars

The pair-wise Jaccard's coefficient for the genetic similarities among the 24 cultivars are presented in Table 4. The similarity coefficient value varied from 0.52 (between Gurmatia and IR-64, Samudrabali and Nariyaphul and Nadiarasa and Gayatri) to 0.94 (between Samudrabali and Gurumatia). The high degree of

Table 3. Amplification patterns of 24 rice varieties with 15 random primers

S.No.	Primer code	No. of loci	Polymorphic loci		Monomorphic loci		Unique loci		Genotypes with unique amplification
			No.	%	No.	%	No.	%	
1	57ss	7	6	65.7	1	14.3	0	0.00	
2	63ST	6	6	100.0	0	0.00	0	0.00	
3	62ss	8	2	25.0	5	62.5	1	12.50	Gopalbhog
4	58ST	4	3	75.0	1	25.0	0	0.00	
5	65ss	4	4	100.0	0	0.0	0	0.00	
6	60ss	5	4	80.0	1	20.0	0	0.00	
7	67ss	8	7	87.5	1	12.5	0	0.00	
8	66ST	6	3	50.0	2	33.3	1	16.70	IR-64
9	68ss	6	4	66.7	2	33.3	0	0.00	
10	69ST	7	4	57.2	3	42.8	0	0.00	
11	E98932	3	3	100.0	0	0.00	0	0.00	
12	E98934	4	4	100.0	0	0.00	0	0.00	
13	E98933	10	4	40.0	6	60.0	0	0.00	
14	E98936	6	5	83.3	1	16.7	0	0.00	
15	E98937	2	1	50.0	1	50.0	0	0.00	

genetic similarity (0.94) among the scented rice cultivars such as Samudrabali and Gurumatia indicated their possible relatedness by descent that might be through selection by local farmers. Nearly similar level of polymorphism and genetic similarity was observed by earlier workers [21, 22] on RAPD profiles of scented rice. The groups of scented and non-scented rice varieties were found to be clearly distinguished from one another in the present RAPD profile, which suggests that RAPD could be a simple and rapid tool for assessing the genetic diversity among different groups of rice cultivars.

Cluster analysis

The cluster analysis based on the similarity coefficient using UPGMA clearly distinguished all the 24 accessions into 3 groups (Fig. 2). The cultivar Nadiarasa and Nariyaphula were grouped together and they were separated from the remaining accessions with less than 60 per cent similarity. All the non-scented rice cultivars were grouped together and they were separated from the remaining group of scented rice cultivars with varied percentage of similarity (nearly 66 to 71%). The two major clusters obtained from the RAPD profile includes Basuabhog, Karpurakanti, Gangabali, Maharajii, Lectimachi, Laxmisiali, Kalajira, Dubraj, Gurmatia and Samudrabali in the first cluster which is separated from the second cluster comprising Maguraphula, Kainchaphula, Samaleibhoga, Mashiphula, Gopalbhoga, Dhusara, Haldilgundi, Tulasibasa, Neela, Lalat, Gayatri and IR-64. The varieties Samudrabali and Gurumatia are grouped together with maximum genetic similarity (0.94) followed by Laxmisiali and Kalajira with

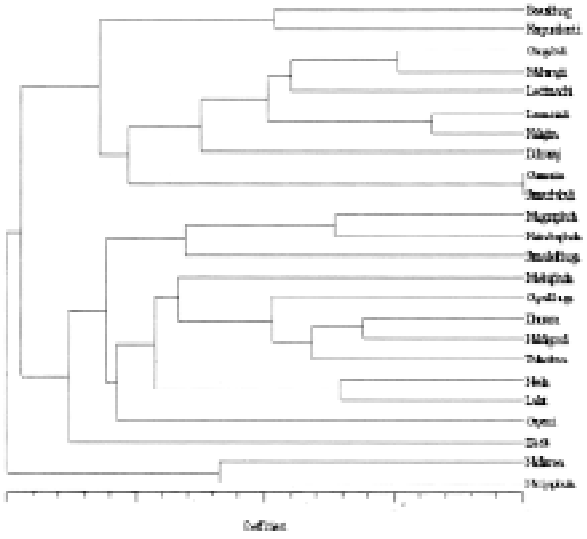

Fig. 2. Dendrogram of 24 rice genotypes based on 15 random primers. The bar at bottom represents similarity index based on Jaccard's coefficients

Table 4. Similarity matrix for Jaccard's coefficients for 24 rice genotypes based on 15 random primers

S.No.	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	Basuabhog	1																							
2	Karpurakanti	0.79	1																						
3	Oangabali	0.74	0.73	1																					
4	Nadiarasa	0.60	0.64	0.73	1																				
5	Nariyaphula	0.58	0.59	0.61	0.75	1																			
6	Maguraphula	0.63	0.65	0.64	0.62	0.65	1																		
7	Kainchaphula	0.71	0.67	0.76	064	0.65	082	1																	
8	Maharajii	0.76	0.72	0.86	0.69	0.60	0.65	0.72	1																
9	Dubraraj	0.59	0.67	0.76	0.65	0.58	061	0.68	0.72	1															
10	Lectimachi	0.66	0.69	078	0.72	0.58	0.61	0.68	0.82	0.79	1														
11	Laxmisiali	068	0.71	0.80	064	0.54	0.60	0.69	0.82	0.74	084	1													
12	Kalajira	0.65	0.66	0.75	0.69	0.61	0.59	0.67	0.74	0.70	0.74	0.88	1												
13	Mashiphula	0.72	0.68	0.72	0.65	0.60	0.69	0.73	0.76	0.59	0.66	0.68	0.70	1											
14	Gopalbhoga	0.64	0.65	0.66	0.62	0.71	0.68	0.70	0.65	0.63	0.66	0.65	0.65	0.77	1										
15	Dhusara	0.65	0.63	0.70	068	0.69	0.67	0.74	0.66	0.67	0.67	0.63	0.66	0.72	0.83	1									
16	Haldigundi	0.65	0.66	0.65	0.64	0.62	0.67	0.72	0.64	0.67	0.65	0.57	0.61	0.73	0.75	0.84	1								
17	Tulasibasa	0.62	0.61	0.65	0.68	0.66	0.69	0.77	0.64	0.62	0.67	061	0.61	0.70	0.77	0.81	0.81	1							
18	Samaleibhoga	0.65	0.66	0.67	0.58	0.66	0.72	0.74	0.69	0.62	0.64	0.66	0.68	0.67	0.75	0.73	0.68	0.70	1						
19	Neela	0.57	0.55	0.66	065	0.60	J0.63	0.65	0.63	0.66	0.64	0.58	0.60	0.74	0.71	0.75	0.70	0.67	0.67	1					
20	Lalat	0.59	0.55	0.64	0.70	0.65	0.60	0.65	0.63	0.63	0.64	0.56	0.60	069	0.71	0.77	0.70	0.72	0.69	0.83	1				
21	IR-64	0.63	0.59	0.64	0.59	0.59	0.57	0.65	0.63	0.59	0.55	0.57	062	0.69	0.66	0.72	0.65	0.64	064	0.68	0.74	1			
22	Gayatri	0.65	0.56	060	0.52	0.54	0.62	0.64	0.64	0.62	0.62	0.59	0.59	0.72	0.72	0.65	0.68	0.65	0.68	0.72	0.69	0.64	1		
23	Gurmatia	0.66	0.65	0.64	0.60	0.54	0.56	0.61	0.70	0.68	0.79	0.74	0.67	0.59	0.63	0.64	0.67	0.69	0.62	0.55	0.59	0.52	0.69	1	
24	Samudrabali	0.67	0.65	0.67	0.61	0.52	0.55	0.61	0.68	0.71	0.77	0.70	0.63	0.58	0.59	0.65	0.68	0.67	0.63	0.55	0.59	0.53	0.65	0.94	1

similarity coefficient (0.88). There was no duplicate accession found in the present study.

The grouping of genotypes into different clusters using molecular marker has great relevance to the scented rice improvement programme. The present study indicates the level of genetic variability present among the local accessions which would be useful for the selection of donors in the development of scented rice varieties. The study also revealed that although RAPD analysis involves considerable amount of effort, time and cost it can be efficiently used for the purpose of studying the genetic diversity among rice cultivars.

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