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Genetic Diversity Analysis of Traditional Aromatic Rice Using Molecular Markers

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Abstract

The study was undertaken to assess the genetic diversity on the basis of molecular characterization among 48 traditional aromatic rice varieties of India. It is very important for germplasm management, varietal identification, and DNA fingerprinting. Twenty four ISSR markers were studied across 48 traditional aromatic rice to characterize and discriminate among different varieties. A total of 151 polymorphic alleles were detected whereas 37 monomorphic alleles were detected. Polymorphic information content (PIC) was found to be the highest in primer (AM-8) and lowest in primer UBC-840. The morphological attributes like panicle number, grain length, no. of fertile grains/panicle and potential yield showed significant variation among the genotype. The morphological attributes are closely resemblance with molecular analysis. Result revealed that the primer AM-8 might be the best marker for identification and diversity estimation of aromatic rice varieties, followed by AM-4, AM-1, UBC-818 and UBC-850 primers. The UPGMA cluster dendrogram created in this study identified two clusters with a similarity coefficient of 53%. The genotype pair ('Dangerbasumati' and 'Gangaballi') showed the maximum similarity (0.93) among the 48 aromatic genotypes. The ISSR polymorphism and diversity could likely be attributed to pedigree. This study offered a rapid and reliable method for the estimation of variability between different traditional aromatic rice varieties which could be utilized by the breeders for further improvement of the aromatic rice varieties.

Key words: Aromatic rice, DNA fingerprinting, ISSR-PCR, Morphological Attributes, Polymorphism,

Abbreviations: PIC- Polymorphic information content, ISSR-Inter Simple Sequence Repeat, CV – Critical variation, UPGMA- Unweighted Pair Group Method based on Arithmetic Average

Introduction

Rice (*Oryza sativa* L.) (2n = 24) belonging to the family Poaceaee and subfamily, Oryzoidea is the staple food for half of the world's population and occupies almost one-fifth of the total land area covered under cereals. It is one of the very few crop species endowed with rich genetic diversity which account over one lakh landraces and improved cultivars. Being the secondary centre of origin of cultivated rice; Odisha has the distinction of possessing about 15,000 traditional rice varieties out of 50,000 found in the world [1]. Among these traditional rice varieties, land races of aromatic rice bear special significance because of their special flavour and economic value in the present globalized era. It is estimated that more than one hundred land races of aromatic rice are found in Odisha [2]. They are mostly short grained with pleasant aroma. Unlike Basmati rice, these varieties retain aroma when grown in prevailing subtropical warm climate of the state. These indigenous aromatic rice genotypes are endowed with tremendous genetic variability and are vital genetic resources for biotic and abiotic stress tolerance, reduction of growth duration and improved nutritional characteristics [3,4,5]. Aroma, length and taste of some of the short grained aromatic rice is known to be superior to Basmati types. Domestic market exists for the indigenous aromatic rice which is popular in their native areas of cultivation. The characterization and improvement of indigenous small and medium grained aromatic rice, which possesses outstanding quality like aroma, kernel elongation after cooking, fluffiness and taste were somewhat neglected as they lacked export value. With growing demand for aromatic rice in international market, high emphasis was placed till now on improvement of basmati types. Some of these genotypes are being gradually eroded from their respective places of origin and are on the verge of becoming extinct due to competition from high yielding varieties, difficulties of cultural practices and improper means of storage [6, 7]. Little attention has been paid to the improvement of short and medium grain aromatic rice except for sporadic reports on germplasm evaluation and genetics of some quality trait. As such, there is very little information available on genetic diversity of traditional short and medium grain indigenous aromatic rice, therefore, these varieties have to be collected and evaluated for their exploitable genetic variability and conserved. The genetic divergence among the aromatic rice genotypes is also a vital tool to the plant breeders for an efficient choice of parents for plant improvement as genetically diverse parents are likely to contribute desirable segregants and/or to produce high heterotic crosses. Further, management of the indigenous aromatic rice genetic resources by way of characterisation and documentation helps in protection of these unique bioresources in accordance with the provision laid out in the 1992 meet on Conservation of Biological Diversity (CBT). Characterization of varieties based on morphological characters is not very reliable because major characters have low heritability and are genetically complex warranting more precise techniques. Several molecular marker techniques are now available. They are more reliable, and remain unaffected across different growth stages,

seasons, locations and agronomic practices [8 , 9]. A random set of these mapped markers providing genome-wide coverage should facilitate an unbiased assay of genetic diversity and thus giving a robust, unambiguous molecular description of rice cultivars [10]. The working group on Biochemical and Molecular Techniques (BMT) of the International Union for the Protection of New Varieties of Plants (UPOV) has in fact identified microsatellite as the most widely used marker system for plant variety characterisation [11].

Unfortunately, there are few reports to date on exhaustive characterization of aromatic rice germplasm using molecular marker in combination with qualitative rice grain characters and agronomic traits. The present investigation is, therefore, aimed at recording the innate phenotypical variations among forty-eight traditional aromatic rice varieties collected from different rice growing pockets of Eastern India and estimating their genetic divergence using the 24 ISSR markers.

Materials and methods

Morphological Characterization

In the present study, forty-eight promising traditional aromatic rice varieties were collected and used for the present experiment. The morphological and agronomical attributes placed in Table 1. These varieties were maintained in germplasm Rice Research Station, Orissa University of Agriculture and Technology, Bhubaneswar; the field trials were carried out in Randomized Block Design with plot size of 3 metre square. Each variety/ treatment is repeated thrice. One month old seedlings were transplanted in a spacing of 20 cm between lines and 10 cm between plants and standard recommended agronomic practices were followed for raising the crop. Observation on different agronomic characters viz. plant height, number of productive tillers/ plant, panicle length, filled grains/ panicle, 100 grain weight, days to maturity, grain yield (kg)/ plot and disease reaction of the genotypes were recorded from five randomly chosen plant/ treatment/ replication. While observation on days to maturity was recorded on plot basis on eye estimation, observation on disease reaction was recorded on actual score basis.

DNA extraction and ISSR analysis

Four week after transplanting, about two grams of leaf samples from each variety was harvested. Total genomic DNA was extracted from the leaf samples by using the modified CTAB method [12]. Twenty one ISSR markers, covering all the 12 chromosomes of rice, were selected from the Genome Databases(http:/ars_genome.cornell.edu/rice/microsats.html).

These primer sequences were custom synthesized by Merck Bioscience, Bangalore, India and were used for the analysis of 48 aromatic rice genotypes. Individual PCR amplifications for each ISSR primer were performed programmable thermal cycler ((BioRad, California, USA)).The PCR protocol involved a total volume of 25 µl reaction mixture containing 40 ng of genomic DNA, 1X PCR buffer (pH 8.3), 200 µM dNTP mix, 10 pmol of each of the forward and reverse primers, 2 mM of MgCl2 and 1 U of Taq DNA polymerase(Merck Bioscience). The amplification was performed in programmable gradient thermal cycler (Bio-Rad, USA) with following programme: a pre-denaturation at 94 °C for 3 min followed by 44 cycles of denaturation at

94 °C for 1 min, annealing at appropriate temperature (55°C or 67°C depending on the primer) for 1 min and 1 min primer elongation at 72°C. The final extension was made at 72°C for 7 min. The amplified PCR products were separated in 2.5 percent agarose gel prepared in 0.5X TBE buffer stained with ethidium bromide. The gel was run in 0.5 X TBE buffer at constant voltage of 90 V for a period of 45 min to 1h. The gel was visualized in UV transilluminator and photographs taken using gel documentation system (Gel Doc. 2000, UVITECH, UK). Amplified polymorphic products from microsatellite analyses were scored qualitatively for presence (1) and absence (0) for each marker allele-genotype combination. The data entry was done into a binary data matrix as discrete variables

Data analysis

Statistical analyses for the morphological and ISSR marker data were conducted using the software NTSYS-pc version 2.1 (Exeter software, Setauket, USA) [13]. The morphological characters were standardized prior to cluster analysis. The categorical (taking a value among many possibilities) values of the state of each morphological character as per the DUS guideline were recorded and subjected to statistical analysis [14]. The matrix of average taxonomic distance for individuals and morphological traits was then computed using SIMIQUAL function and EUCLIDIAN distance coefficient. This similarity coefficient is based on categorical data collected for the morphological traits. Cluster analysis was then conducted on the taxonomic distance matrix with the Unweighted Pair Group Method based on Arithmetic Average (UPGMA) and a dendrogram was generated based on the genetic distance matrix. For analyses based on ISSR markers data from all the markers were used to estimate the similarity on the basis of the number of shared bands. Similarity was calculated with SIMQUAL function of NTSYS that computes a variety of similarity and dissimilarity coefficients for qualitative data. The similarity matrix values based on Jaccard's coefficient of similarity were calculated. The similarity matrix thus generated was used to generate dendrogram based on UPGMA. In order to estimate the congruence among dendrograms, cophenetic matrices for which marker and index type were computed and compared using the Mantel test. Principal component analysis was performed in order to highlight the resolving power of the ordination.

Polymorphic information content that provides an estimate of the discriminatory power of a locus or loci, by taking into account not only the number of alleles that are expressed, but also relative frequencies of those alleles, was estimated using the formula suggested by Powell et al. [15] and Smith et al. [16].

$$PIC = 1 - \sum_{i=1}^{n} f^2_{ij}$$

Where *fij* is the frequency of j^{th} allele for marker ***i*** and the summation extends over ***n*** alleles.

Pearson's correlation coefficients (r) for kernel length, kernel breadth, l/b ratio, test weight, aroma, presence of awn, days to 50% flowering, plant height, productive tillers per plant, length of panicle, filled grains per panicle and presence of pubescence on lemma palea were calculated using SPSS software.

Results

The morphological characteristics of 48 traditional aromatic rice varieties are presented in Table.1. The grain morphology of 48 varieties were depicted into three groups such as very short length, short and medium length. Out of the 48 verities, 10 having very short length grain (< 5.52 mm), 10 varieties short grains (5.55 – 6.84 mm) and twenty eight having medium grain (6.85 – 8.17mm). There was a significant variation in grain colour among the 48 varieties. Five varieties (Nuakalajerra, Jalaka-2, Kalajera, Khosakani and Ganjam Local-1) having black coloured grain, nine varieties (Basnasapuri, Basnaparijat, Sujata, Srimula, Tulasiphulla, Saragadhulli, Ganjeikalli, Kaminibhoga-2) having gold and brown grain and one variety (Sujata) red colour and others were brown coloured grains. The grain width was categorized into three group i.e. medium (1.74 – 2.36 mm), broad (2.37 – 3.0 mm)and very broad (> 3 mm). The yield potential, 1000 grain weight, kernel length of 48 varieties of aromatic rice ranged from 10.49 to 29.42 quintals per hectare, 10.70 – 19.20 g and 2.90 – 6.10 mm respectively (Table 1). The grain morphology varied considerably in 48 varieties with respect to awnness, colour and size of awns, lemma and palea with presence or absence of coloured furrows and spots, pubescence were presented in Fig.1. The kernel breadth, a quality determinant, among the varieties revealed a major variation ranging from 1.20 mm ('Pimpudibasa') to 3.2 mm ('Gatia'). The minimum kernel ratio was recorded in variety 'Gatia' (1.28 l/b), while maximum exhibited in variety "Sujata" (3.28 l/b). The agronomical characters for 48 varieties are recorded with respect to days to 50% flowering, height of the plant, the average number of productive tillers per plant, average panicle length ranged and the mean number of filled grains per panicle. The minimum number panicle (6.0) was recorded in variety 'Jaiphulla', 'Jalala-1", 'Manasi-1', 'Mugajai', 'Tulasiphada', Krishnabhoga', 'Basumatidhan' and maximum (13.0) in 'Kalikati-2'. As far as variation in the agronomic traits is concerned, the panicle number recorded high variation (%CV = 10.66) and average number of filled grains recorded lowest variation (%CV = 9.83). The twenty four ISSR markers were used to characterize and assess the genetic variability among 48 aromatic rice genotypes. All the ISSR primers showed polymorphism among 48 aromatic rice varieties (Fig. 2a-d). ISSR primers yielded total of 151 bands ranging in size from 150 bp to 2900 bp among the forty eight genotypes. The ISSR primer UBC-811 produced the maximum 9 bands followed by AM-1 (8 bands). The lowest number of bands was observed in UBC-818 (two bands) and the average number of bands per primer was found to be 6.29. Of a total 151 bands, 114 (75.5 %) were found to be polymorphic while 37 bands (24.50 %) were monomorphic. The average number of polymorphic bands was 4.75 per primer. The highest percent polymorphism was 100% (Table 2). Polymorphism information content (PIC) was found to be the highest in AM-8 (0.884) and the average PIC content of twenty four ISSR primers among all forty eight aromatic genotypes was found to be 0.51. The DNA profile data derived from ISSR primers were subjected to calculate the genetic similarity and the matrix index. The similarity matrix used to determine level of

relatedness among the aromatic rice genotypes studied. Pair-wise estimates of similarity matrix ranged from 0.39 to 0.93 and average similarity among all 48 genotypes was 0.56 (Fig.3). The maximum numbers of genotypes (43) were represented in Cluster I whereas five genotypes viz. 'Kalikati-2', 'Kanakachampa', 'Kusumbhog', 'Manasi-1' and 'Karpurkrati' were placed in Cluster II. Cluster I was divided into sub-cluster 'IA' and sub-cluster 'IB' at 60% similarity coefficient. Cluster 'IA' was represented by 24 genotypes and Cluster 'IB' had 18 genotypes. Sub-cluster 'IA' was further divided into 'IA_1' and 'IA_2'at 61% similarity coefficient. Sub-sub-cluster 'IA_1' included the aromatic rice varieties namely 'Bananangemati', 'Dhobalachi', 'Basnapuri', 'Basnaparijit', 'Basumati', 'Basumati dhan' were included while the sub-cluster 'IA_2' was represented by 18 aromatic rice varieties. The cluster 'IB' was further divided into 'IB_1' and 'IB_2' at 59% similarity coefficient. The sub-cluster 'IB_1' contained 17 genotypes whereas a single variety 'Kalikati-1' was placed in'IB_2'. The data generated using twenty four ISSR primers were used in PCA analysis using Jaccard's similarity coefficient (Fig.4.). The two-dimensional scaling of PCA analysis placed all the 48 aromatic rice varieties into four groups. First group contain 5 genotypes such as 'Kalikati-2', 'Kanakachamp', 'Kusumabhoga', 'Karpurakranti', 'Manasi-1'. Second group contain one variety 'Kalikati-1.' Third group having 18 genotypes i.e. 'Dholabankoi', 'Dubraj', 'Dulhabhoga', 'Dangarabasamati', 'Gangaball', 'Ganjam local-1', 'Ganjam local-2', 'Gatia', 'Ganjeikalli', 'Heerakani', 'Jalaka-1', 'Jalaka-2', 'Jaiphulla', 'Kalajeera', 'Kalajauvan', 'Khosakani', 'Kaminibhoga-1' 'Kaminibhoga-2' and fourth group having 24 genotypes placed separately (Fig.3). The variety 'Kalikati-1' alone was placed away from the rest of the varieties.

Table.1 Morphological characteristics of aromatic rice genotypes.

SLNo.	Genotypes	Panicle Number	No of Fertile Grains/panicle	1000 grain weight (g)	Potential Yield(q/ha)	Grain length(L) (mm)	Grain width(W) (mm)	Grain (L/B) ratio	Kernel length (mm)	Kernel breadth (mm)	Kernel (L/B) ratio
1	2	3	4	5	6	7	8	9	10	11	12
1	Baranamgomati	8	97	17.8	22.61	7.1	3.4	2.09	5.1	2.1	2.43
2	Basnasapuri	11	173	11.0	25.07	5.8	1.8	3.22	4.8	1.9	2.53
3	Basnaparijat	10	147	11.1	22.22	6.1	2.1	2.90	4.8	1.9	2.53
4	Basumati-1	9	62	13.9	23.95	5.6	1.8	3.11	4.5	2.1	2.14
5	Basmatidhan	6	116	12.8	18.62	6.1	2.1	2.90	4.1	2.2	1.86
6	Basumati Bhog	7	195	13.3	27.36	6.8	1.9	3.58	4.1	1.5	2.73
7	Chatianaki	7	123	12.5	19.59	6.3	2.8	2.25	4.9	1.5	3.27
8	Dhobaluchi	7	123	14.4	27.46	5.9	2.9	2.03	3.9	2.5	1.56
9	Krishnabhoga	6	128	13.1	25.22	6.9	3.1	2.23	5.1	2.1	2.43
10	Manasi-2	9	117	13.2	23.48	4.9	1.8	2.72	4.1	1.5	2.73
11	Nuakalajeera	7	159	11.0	20.78	5.1	2.2	2.32	3.1	1.2	2.58
12	Nuadhusura	8	110	15.9	20.72	5.8	1.9	3.05	4.1	1.5	2.73

13	Pimpudibasa	6	160	13.3	18.57	5.1	2.2	2.32	2.9	1.2	2.42
14	Ratnasundari	10	118	14.5	19.64	4.9	2.1	2.33	3.5	1.8	1.94
15	Sujata	9	140	14.1	26.32	8.2	2.1	3.90	5.9	1.8	3.28
16	Srimula	8	131	11.1	21.59	6.2	2.7	2.30	4.9	2.5	1.96
17	Thakurbhog	10	145	12.9	28.12	6.1	2.8	2.18	5.2	2.5	2.08
18	Tulasiphada	6	126	13.8	24.71	6.1	3.1	1.97	4.8	2.3	2.09
19	Sheetabhog	8	145	10.7	19.13	6.2	2.3	2.70	5.5	1.9	2.89
20	Gopalabhoga	7	125	11.5	18.57	6.2	3.1	2.00	4.2	2.5	1.68
21	Tulasiphulla	9	108	13.7	10.49	6.3	2.5	2.52	4.1	2.3	1.78
22	Bhasumati (P)	8	125	12.4	23.54	6.4	3.4	1.88	4.5	2.5	1.80
23	Saragadhulli	10	132	11.8	19.23	5.9	2.9	2.03	3.9	2.3	1.70
24	Mugajai	6	153	17.5	22.45	5.9	3.1	1.90	4.1	2.3	1.78
25	Dholabankoi	9	169	12.4	29.42	6.2	3.3	1.88	4.5	2.3	1.96
26	Dubraj	8	152	14.5	26.37	8.2	2.3	3.57	6.1	2.1	2.90
27	Dulhabhoga	7	141	14.9	17.65	5.1	1.9	2.68	4.2	1.9	2.21
28	Dangarabasamati	7	135	13.6	19.24	5.9	2.1	2.81	4.5	1.8	2.50
29	Gangaballi	9	139	11.6	24.48	5.9	1.9	3.11	4.2	1.5	2.80
30	Ganjam local-1	8	137	11.8	19.20	6.1	2.9	2.10	4.2	2.3	1.83
31	Ganjam local-2	7	128	13.9	27.29	5.9	1.9	3.11	4.1	2.1	1.95
32	Ganjeikalli	10	130	18.3	25.42	6.9	3.3	2.09	4.2	3.1	1.35
33	Gatia	8	135	18.2	24.37	6.9	3.5	1.97	4.1	3.2	1.28
34	Heerakani	10	175	11.9	23.16	5.9	2.5	2.36	4.5	2.2	2.05
35	Jalaka-1	6	112	14.2	22.81	4.9	2	2.45	3.9	1.5	2.60
36	Jalaka-2	10	136	11.6	18.71	5.1	1.9	2.68	4.1	1.4	2.93
37	Jaiphulla	6	107	19.2	25.68	5.9	1.9	3.11	4.1	1.5	2.73
38	Kalajeera	8	123	14.4	25.55	5.2	2.1	2.48	3.1	1.7	1.82
39	Kalajauvan	7	143	13.5	18.61	5.1	1.8	2.83	3.1	1.6	1.94
40	Khosakani	7	174	13.9	28.41	7.1	3.1	2.29	5.1	2.5	2.04
41	Kaminibhoga-1	6	163	14.6	20.31	6.1	2.9	2.10	5.1	2.5	2.04
42	Kaminibhoga-2	7	145	11.9	19.77	6.9	2.5	2.76	6.1	2	3.05
43	Kalikati-2	13	174	12.3	24.07	6.2	2.8	2.21	5.5	2.5	2.20
44	Kalikati-1	7	164	10.9	21.39	8.1	3.2	2.53	6.1	2.5	2.44
45	Kanakachampa	7	146	12.1	18.62	7.1	2.9	2.45	4.1	2.5	1.64
46	Kusumabhoga	12	138	13.6	19.42	5.9	3.5	1.69	4.2	2.5	1.68
47	Karpurakranti	7	88	13.6	17.65	6.9	2.3	3.00	4.2	2.3	1.83
48	Manasi-1	6	72	15.1	19.67	7.1	2.4	2.96	4.1	2	2.05
	TOTAL	384.00	6484.00	649.30	1066.71	296.50	121.00	121.65	213.50	99.40	106.74
	Mean	15.67	264.65	26.50	43.54	12.10	4.94	4.97	8.71	4.06	4.36
	Maximum	13.00	195.00	19.20	29.42	8.20	3.50	3.90	6.10	3.20	3.28
	Minimum	6.00	62.00	10.70	10.49	4.90	1.80	1.69	2.90	1.20	1.28
	SD	1.67	26.00	2.03	3.69	0.81	0.55	0.50	0.75	0.45	0.49
	CV	10.66	9.83	7.65	8.47	6.68	11.04	10.05	8.62	11.09	11.18

Table.2. DNA profile and polymorphism generated in 48 Aromatic rice genotypes using 24 ISSR primers

Sl. No.	Primer Code	Sequence 5'-3' ⟶	Tm (°C)	Total No. of bands (bp)	Monomorphic band (bp)	Polymophic band (bp)	% of Polymorphism	Size Range (bp)	Average PIC value
1	AM-1	$(GGC)_5AT$	58ºC	8	3	5	62.50	380-1100	0.778
2	AM-2	$(AAG)_5GC$	38ºC	6	0	6	100.00	410-990	0.577
3	AM-3	$(AAG)_5TG$	38 ºC	6	1	5	83.33	600–2700	0.736
4	AM-4	$(AAG)_5CC$	40ºC	7	2	5	71.43	310-950	0.853
5	AM-5	$(AGC)_5CA$	57 ºC	6	0	6	100.00	500–2000	0.641
6	AM-6	$(AGC)_5CG$	50 ºC	7	1	6	85.71	750–2900	0.587
7	AM-7	$(GGC)_5TA$	58 ºC	6	0	6	100.00	700–2850	0.543
8	AM-8	$(AGC)_5GA$	53 ºC	7	2	5	71.43	650–2600	0.884
9	AM-9	$(AAG)_5CG$	40 ºC	7	2	5	71.43	650–2800	0.601
10	AM-10	$CCA(GTG)_4$	45 ºC	7	1	6	85.71	650–2300	0.623
11	UBC-807	$(AG)_8T$	34ºC	4	1	3	75.00	350-1550	0.458
12	UBC-808	$(AG)_8C$	37 ºC	6	3	3	50.00	300-1880	0.432
13	UBC-809	$(AG)_8G$	37 ºC	7	4	3	42.86	250-2000	0.357
14	UBC-811	$(GA)_8C$	45ºC	9	0	9	100.00	200-1200	0.749
15	UBC-812	$(GA)_8A$	38 ºC	7	4	3	42.86	300-1800	0.458
16	UBC-813	$(CT)_8TT$	36ºC	6	0	6	100.00	400-1000	0.688
17	UBC-818	$(CA)_8AG$	36ºC	2	0	2	100.00	350-1500	0.749
18	UBC-825	$(AC)_8T$	34ºC	7	2	5	71.43	250-1800	0.307
19	UBC-840	$(GA)_8YT$	46ºC	7	5	2	28.57	200-1400	0.345
20	UBC-842	$(GA)_8YC$	45 ºC	6	2	4	66.67	300-1800	0.689
21	UBC-843	$(CT)_8RA$	38 ºC	7	3	4	57.14	250-1850	0.548
22	UBC-850	$(GT)_8YC$	42ºC	6	1	5	83.33	150-1900	0.704
23	UBC-864	$(ATG)_6$	38ºC	6	0	6	100.00	200-1950	0.394
24	UBC-872	$(GATA)_4$	38ºC	4	0	4	100.00	200-2100	0.639
	Total			151	37	114			

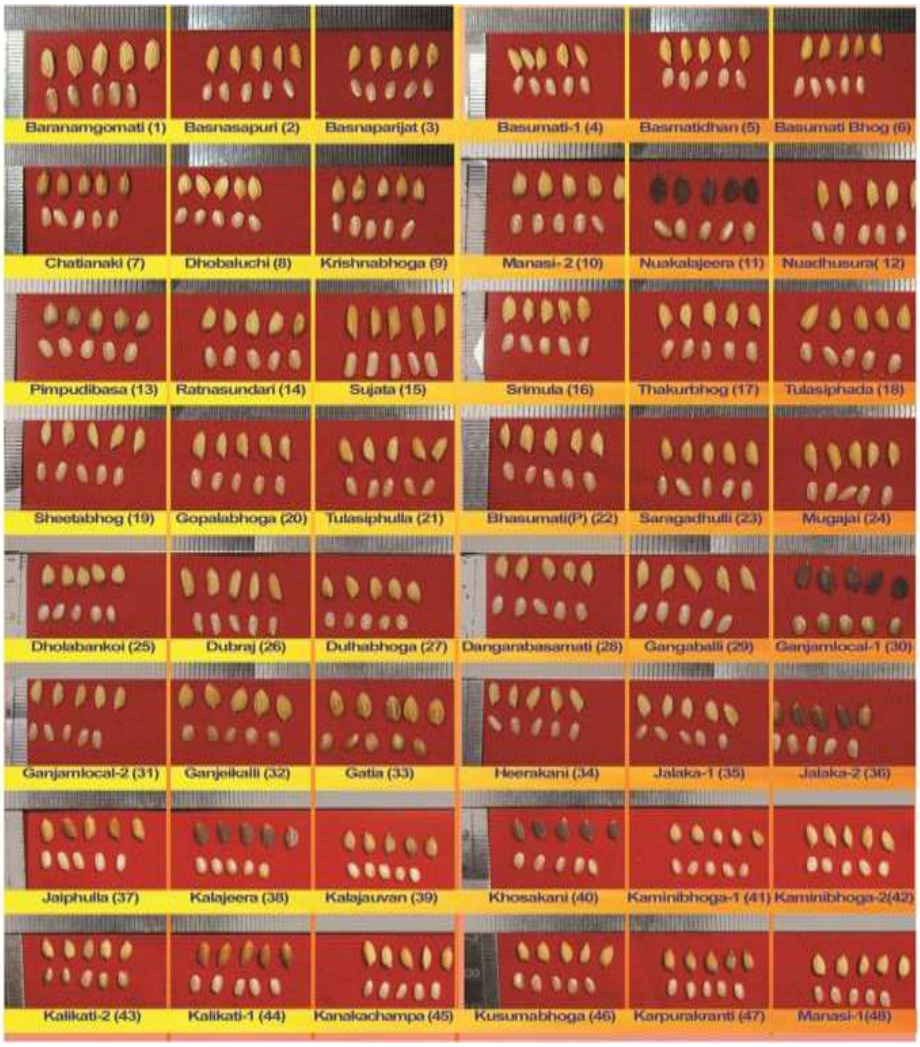

Fig. 1. Morphology of whole and dehusked rice grain of 48 Aromatic rice varieties

Discussion

The grain morphology varied considerably in 48 varieties with respect to awnness, colour and size of awns, lemma and palea with presence or absence of coloured furrows and spots, pubescence. Hien et al. [17] reported that in aromatic rice cultivars from Asia morphological traits were useful for preliminary evaluation and could be used as general approach for assessing genetic diversity among morphologically distinguishable aromatic rice cultivars. Patra and Dhua [18] reported that the less variation in morphological characters among 120 accessions of upland rice collected from Jaypore tract of Orissa. The kernel breadth, a quality determinant, among the varieties revealed a major variation ranging from 1.20 mm ('Pimpudibasa') to 3.2 mm ('Gatia'). The minimum kernel ratio was recorded in variety 'Gatia' (1.28 l/b), while maximum exhibited in variety "Sujata" (3.28 l/b). Classical breeding affects genetic diversity within breeding programs. Selection increases the frequency of alleles or allelic combinations with favourable effects at the expense of others, eventually eliminating many of them [19]. In the present investigation, twenty four ISSR markers were used to characterize and assess the genetic variability among 48 aromatic rice genotypes. All the ISSR primers showed polymorphism among 48 aromatic rice varieties. ISSR primers yielded total of 151 bands ranging in size from 150 bp to 2900 bp among the forty eight genotypes. Of a total 151 bands, 114 (75.5 %) were

found to be polymorphic while 37 bands (24.5.0 %) were monomorphic. Larger number of polymorphic markers generated by ISSR can be attributed to the fact that the centromeric region contains a large amount of repeated sequences, highly polymorphic due to DNA slippage [20, 21, 22]. The DNA profile data derived from ISSR primers were subjected to calculate the genetic similarity and the similarity matrix. Pair-wise estimates of similarity matrix ranged from 0.39 to 0.93 among all 48 genotypes.

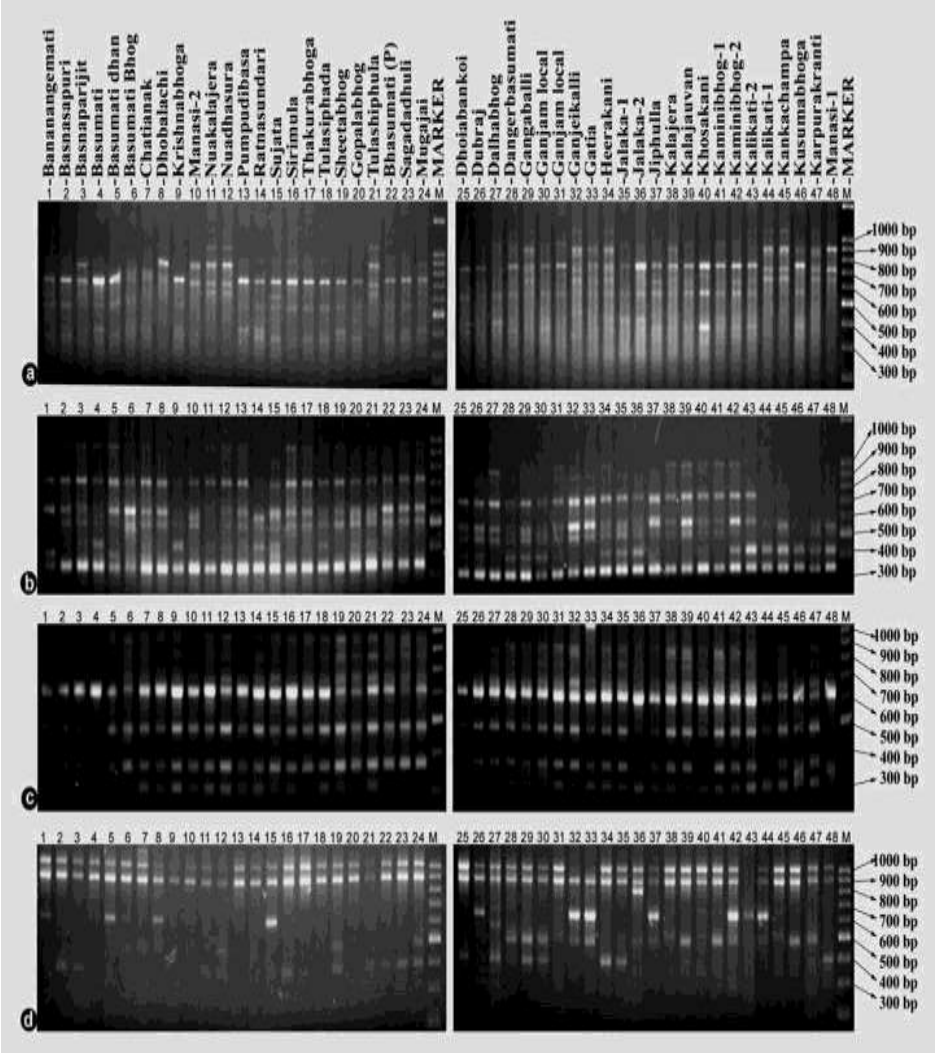

Fig. 2. Amplification profile of 48 aromatic rice genotypes employing ISSR primers
(a) AM-1; (b) AM-2; (c) AM-4 and (d) UBC-813
M: Medium range DNA ruler, Numbers on the margin represent molecular weight of Ruler DNA in base pairs (bp). Numbers on the top of the lanes correspond to the aromatic rice genotypes as given in Table 1

The ISSR amplification pattern was used to establish the genetic variability among the genotypes by cluster analysis and to detect the varietal diagnostic markers. The maximum numbers of genotypes (43) were represented in Cluster I whereas five genotypes were placed in Cluster II. The two-dimensional scaling of PCA analysis placed all the 48 aromatic rice varieties into four groups. The differences in ISSR profile was perhaps due to their adaptability and other significant agronomic characteristics like yield potential, panicle type and seed morphology,

photosynthesis efficiency, grain characteristics. The results suggest that the use of different ISSR primers would enable to assess the genetic diversity of rice as reported previously [23].

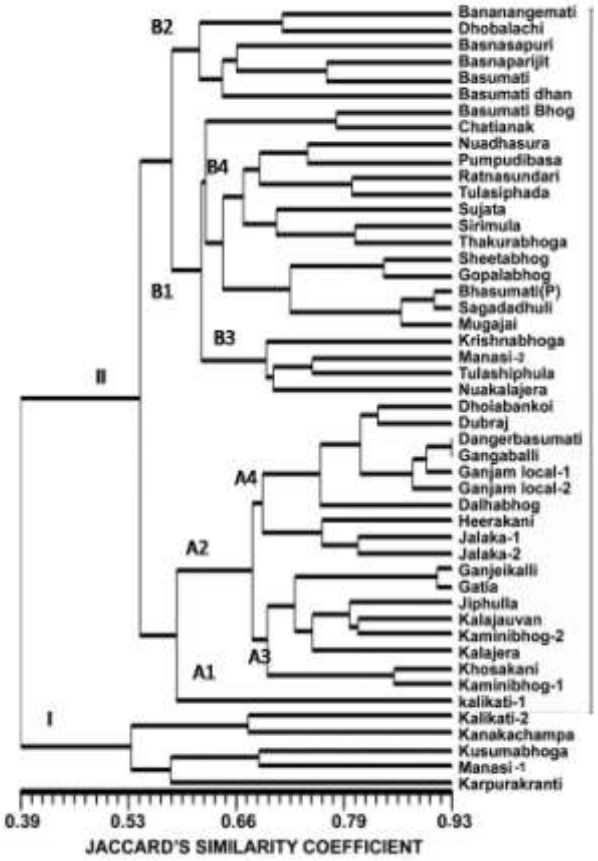

Fig.3. Dendrogram depicting genetic relationship among 48 Aromatic rice varieties based on the ISSR profile

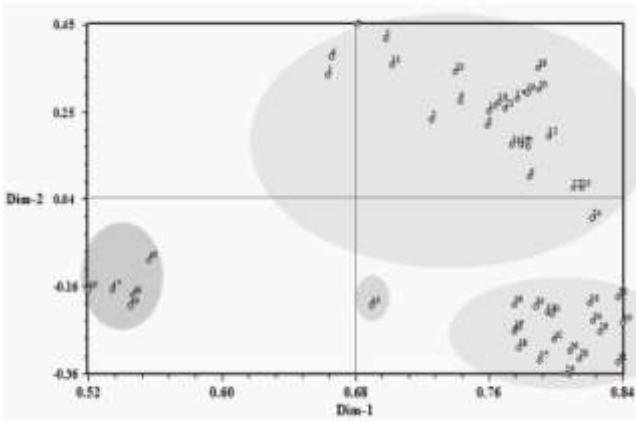

Fig.4. Two Dimensional scaling by Principle Component Analysis (PCA) of 48 Aromatic rice varieties using Jaccard's Similarity Coefficient of ISSR profile data

Molecular markers showed better resemblance with the pedigree as compared to morphological markers. Patra and Chawla [24] reported that the molecular markers help to establish the distinctiveness of Basumati rice varieties. Genetic variation is important in maintaining the developmental stability and biological potential of the genotype. The results indicate that there was very close variation among the varieties. The result suggests that the use of different molecular markers would enable to assess the genetic diversity of aromatic rice variety as reported earlier in other variety of rice [23]. Joshi *et al*. [25] studied the genetic diversity and phylogenetic relatedness in *Oryza* by ISSR markers. The present study showed that the higher percentage of polymorphism as compared with other molecular marker as reported earlier [26]. Bhuyan *et al* [22] illustrated the genetic diversity in traditional lowland rice grown in Assam using both RAPD and ISSR markers. Further, Youssef *et al* [27] used both RAPD and ISSR markers to identify the new promising drought tolerant lines of rice under drought stresses. These traits with molecular differences commented upon in this investigation suggest that these rice varieties were belong to indica rice with introgressions from wild rice land races. The intra and inter genetically variation might be useful for breeders to improve the aromatic rice through selective breeding and cross breeding programs. The ISSR protocol can be readily used in breeding activities for registration and characterization of even closely related rice accessions, and for cataloguing collections. Cluster analysis based variability assessment in rice is reported by several workers [17, 28, 29]. Ratho [30] reported that clustering pattern did not follow the geographical origin of a variety. Sarawgi and Bhisne [31] reported separation of varieties on the basis of agro-morphological and quality characters. Naik *et al*. [29] revealed clustering pattern for 50 scented rice. For higher variability breeding, parent selection based on wider inter-cluster distances [32, 33, 34]. The kernel breadth showed positive association with test weight and negative association with l/b ratio. The kernel l/b ratio was found positively associated with test weight. Among the agronomic traits, plant height exhibited significantly positive correlation with length of panicle and negative correlation with productive tillers per plant and filled grains per panicle. The kernel length exhibited significantly positive correlation with productive tillers per plant and was negatively correlated with filled grains per panicle. The kernel breadth showed positive correlation with plant height and negative correlation with productive tillers per plant. The kernel l/b ratio was found to be positively associated with productive tillers per plant and negatively associated with plant height filled grains per panicle. The test weight showed negative correlation with filled grains per panicle. Similar correlations were reported earlier [35, 36, 37]. ISSR markers are useful towards marker-assisted selection, linkage mapping and to widen the genetic base.

In conclusion, the present investigation provides the guidelines for the selection of parents based on agronomic traits with special reference to qualitative characters for rice improvement program.

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