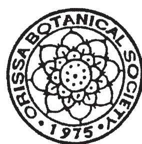

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Analysis of genetic diversity in submergence introgression varieties of different duration rice (*Oryza sativa* L.) of Odisha through RAPD markers

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A B S T R A C T

Genetic diversity of ten rice (*Oryza sativa* L.) varieties of different duration was analyzed through agronomic traits along with RAPD markers. Mid-Early (IR87439, Ciherang *Sub1*, Lalat), Medium (IR88228, Swarna *Sub1*, Pratikshya), and Late duration (IR85086, Savitri *Sub1*, Mahanadi) varieties were tested with a check variety (Swarna) which revealed variation in flowering duration (85 d in Lalat to 110 d in IR85086), plant height and panicle length. 100-grain weight was ranged from 1.92 g in Swarna) to 3.79 g in IR88228. A significant positive correlation of 0.812 was noticed between plant height and leaf area. Plant height, panicle length, flag leaf area have significant positive correlation with 100-grain weight. Out of the 60 RAPD primers, 31 primers produce 280 amplicons (150 to 1960 bp) with a mean 57.24 % polymorphism. Dendrogram obtained from RAPD markers revealed that all the Mid-Early and Medium duration varieties formed Cluster-I except Swarna *Sub1* and the rest of the Late duration varieties formed Cluster-II with check variety Swarna. The PCA analysis confirmed that IR88228, IR87439, Ciharang *Sub1*, and Pratikshya have close genetic similarities as compared to Lalat. Thus, RAPD markers could be used in the identification of varieties and seed certification in rice breeding programs.

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1. Introduction

Oryza sativa L. commonly known as rice belongs to the family Poaceae is one of the most important commercial food grains in the world. High temperature and humidity with high rainfall during the monsoon season favor rain-fed crop cultivation of rice in India. Out of about 162.06 million hectares' total area of rice cultivated in the world, India covers an area of 43.79 million hectares (Anonymous, 2020) under the total irrigated and rain-fed area at present. India accounts for the second-highest production in world of 118.87 million metric tons of milled rice which follows 146.73 million metric tons of rice produced by China. A large number of rice landraces compatible with varied agro-ecological conditions have been evolved and a number of submergence introgressed varieties of rice have been developed in India are being cultivated in the country. A number of such varieties have been cultivated in Odisha conditions to check the yield potential of rice submergence introgressed rice varieties with the non-introgressed landraces of different duration of maturity. It is very much essential to explore the genetic variability of these indigenous rice varieties for better yield and adaptability without replacing indigenous cultivars and landraces. The selection of plant varieties based on morphological characters is sometimes biased with environmental interference and sometimes not reliable because major characters of interest possess low heritability and are genetically complex. Several attempts have been made to synthesize heterogeneous populations of rice taking into account different morphological and yield attributes to segregate the bulk population (Das, 2013, 2018). The influence of environmental factors and nearly similar morpho-characters are the serious concern to distinguish genetically close varieties. Thus, DNA markers are found to be more reliable (Hadrys *et al.*, 1992; Bowditch *et al.*, 1994; Raghunathachari *et al.*, 2000). RAPD, a PCR-based technique

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are used extensively in gene mapping of rice (Welsh and McClelland, 1990; Williams *et al.*, 1990; Mackill, 1995) besides the use of RFLP (Restriction fragment length polymorphism) markers (Nakano *et al.*, 1992; Zhang *et al.*, 1992; Ishii *et al.*, 1993) which have been used in DNA fingerprinting studies. The use of RAPDs has been applied besides restriction fragment pattern of chloroplast DNA (Dally and Second, 1990) in the classification of Indian and Japonica rice cultivars (Zheng *et al.*, 1991; Fukuoka *et al.*, 1992; Yu and Nguyen, 1994). Molecular markers provide information to estimate close relatives and the phylogenetic position of the varieties. DNA fingerprinting based on RAPD markers found varietal distinctiveness and relativeness unambiguously in rice from time to time for salt-tolerant local and inbred rice (Mazumder *et al.*, 1999), non-aromatic rice (Rahman *et al.*, 2007; Joshi *et al.*, 2012; Rajani *et al.*, 2013; Alam *et al.*, 2014; Singh and Sengar, 2015; Karande *et al.*, 2017), coarse and fine grain rice (Arshad *et al.*, 2011), traditional glutinous rice (Shaptadvipa and Sarma, 2009), and aromatic rice (Baishya, 2000; Hasan and Raihan, 2015; Zakiyah *et al.*, 2019). However, the study on genetic variation of submergence tolerant and landraces of different duration of rice varieties of Odisha is scanty. Thus, examination of genomic variation is especially useful for quick varietal genetic variability through RAPD markers accompanying yield attributes for analysis of inherent genetic differences among the individuals for use in breeding programs and introduction of submergence tolerant rice besides the traditional landraces. Keeping all these in purview the present work was undertaken to estimate genetic variation in the germplasm of Mid-Early, Medium and Late duration rice varieties using yield attributes and RAPD markers for future use in selection, hybridization, biodiversity assessment, evaluation and conservation of diverse gene pools of Odisha.

2. Materials and methods

2.1 Plant materials

Ten rice varieties of Odisha were grown in an on-field trial for the study of adaptive variations during Kharif season in the year 2020 in the experimental field of Orissa University of Agriculture and Technology, Odisha, India. The materials were grown in randomized block design, each accession grown in the plant to plant spacing 15 × 15 cm and between-row spacing of 20 cm. General agronomic procedures were practiced at various stages of crop growth and development. Well-rotted Farm Yard Manure (4.0 tones ac^{-1}) was applied 4 to 6 weeks before seed sowing and 10 kg ZnSO4 ac^{-1} during the last puddling stage. Basal fertilization of NPK (0.5:0.5:0.5) kg^{-1} 100 sq. m was applied for robust seedling growth and NPK (100:60:60) was applied in three splits (at 30 days after transplantation, at active tillering stage, and at panicle initiation stage) in field condition. Observations of all the quantitative characters were recorded (Table 1).

2.2 Agronomic parameters

The morphological and yield attributing characters of ten varieties of rice were recorded by selecting randomly three plants in each replication for each variety. Observation on days to 50% flowering and days to maturity was recorded on a pilot basis. The agronomic characters collected have been presented in Table 2. Plant height was measured in cm from the ground level to the tip of the longest panicle of all tillers of the main stem at the time of the harvest. Flag leaf length and breadth were obtained from the mature plants during harvest in cm and the area was calculated. Six randomly selected plants from each plot were harvested and numbers of panicles and panicle lengths were measured. The mean length of all the panicles of a plant was measured and the average was taken. The number of fertile seeds per panicle of each variety was recorded and the fertility percentage was also obtained. The weight of 100 grains of individually selected plants was recorded randomly from each replicated plot and weighed separately and the average was taken.

2.3 DNA extraction and quantification

Nuclear DNA was isolated for each variety from young leaves following the CTAB method (Saghai-Maroof *et al.*, 1984). Leaves were ground to a fine powder in liquid nitrogen using mortar and pestle and suspended in six-volume of CTAB extraction buffer [100 mM Tris HCl (pH 8.2), 20mM EDTA, 0.5M NaCl, 2% CTAB, and 2% beta-mercaptoethanol]. The suspended rice leaf powder in CTAB buffer was incubated in a water bath at 60 ^{o}C for 2 h. The solution was cooled down to room temperature and an equal volume of chloroform: isoamyl alcohol was added to it. The mixture was emulsified for 15 min and centrifuged at 10, 000 rpm for 30 min. The aqueous layer was transferred into a sterilized centrifuge tube and DNA was precipitated with equal volume of pre-chilled isopropanol (kept at 4 ^{o}C for overnight). The thread of DNA was collected with a sterile plastic loop or precipitate was centrifuged at 10,000 rpm for 10 min and the supernatant was discarded. DNA was washed in 70% ethanol and was dried in a vacuum concentrator. Dried DNA samples were dissolved in $T_{10}E_1$ (10 mM Tris HCl, 1 mM disodium EDTA, pH 8.0) and treated with RNase A (10 mg ml^{-1}) and proteinase K solution. The DNA was re-precipitated with an equal volume of ethanol after mixing 3 mM sodium acetate, air-dried, and dissolved in $T_{10}E_1$ buffer. The concentration of DNA was checked in 0.8% agarose gel with lambda DNA as standard in the Gel Doc system. The DNA was diluted into a final concentration of 25 ng μl^{-1} using $T_{10}E_1$ buffer for use as a template during PCR amplification with different primers (Table 5).

2.4 PCR reaction and RAPD analysis

RAPD profiles were generated by using 60 ten base Operon Primers of series A, B, and C having 20 primers each in a polymerase chain reaction (PCR) following the standard protocol. Each polymerase chain reaction (PCR) mixture (25 μl) was prepared with 25 ng of template DNA, master mix having 200 μM of each dNTPs, 0.5 unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), 25 ng of primer, and 10 × PCR assay buffer (50 mM KCl, 10 mM Tris HCl, 1.5 mM $MgCl_2$, pH 9.0). The reaction was performed in a thermal cycler (GENEAMP-9700; Applied Biosystems, USA) with the program having initial denaturation at 95 °C for 5 min, followed by 44 cycles of denaturation (94 °C for 1 min), annealing (42 °C for 1 min), and extension (72 °C for 2 min) followed by a final extension at 72 °C for 8 min. The amplification products after completion of PCR reaction were stored at 4 °C. The amplified products were separated in 1.5% agarose gel containing 0.5 μg ml^{-1} ethidium bromide in TAE buffer (40 mM Tris-acetate, pH 8.0; 2 mM EDTA) at a constant 55 V for 60 to 80 min. A gel loading buffer [20 % (w/v) sucrose; 0.1 M EDTA, 1.0 % (w/v) SDS; 0.25 % (w/v) bromophenol blue; 0.25 % (w/v) xylene cyanol] was used as a tracking dye. Amplified fragments of DNA were observed on a 1.2% agarose gel under a gel documentation system (Geldoc XR system, BioRad, USA) after staining with ethidium bromide solution and photographed. Gene ruler 100 bp DNA ladder was used as marker DNA to determine size of the amplified DNA fragments. Constantly appeared amplification products were used for scoring and further analysis.

2.5 Statistical analysis

Mean data, standard deviation and standard error were calculated for all the replicated agronomic parameters. ANOVA analysis was done among different agronomic parameters. The mean data of yield attributing characters collected against all the varieties of rice were used as a set of variables for the similarity matrix. The correlation coefficient was calculated using Excel Program. The presence or absence of the bands was scored as present (1) or absent (0) in each variety for each set of primers for RAPD analysis. RAPD profile was scored in a binary matrix which was used for the analysis of Jaccard's coefficient matrix (Jaccard 1908). A dendrogram was obtained, from this matrix, by cluster analysis following UPGMA using NTSYSpc version 2.11s software (Rohlf 2008).

3. Results

3.1 Variation in agronomic characters

The agronomical characters of ten studied rice varieties have been given in Table 1. Duration of all varieties of rice was classified as Mid-Early (IR87439, Ciherang *Sub1*, Lalat), Medium(IR88228, Swarna *Sub1*, Pratikshya), and Late duration[IR85086, Savitri *Sub1*, Mahanadi] and Swarna (the check variety). The range of mean plant height varied from 89.53 cm in Swarna *Sub1* to 120.25 cm in IR85086 (Table 2). The duration of the flowering differed in the ten studied varieties of rice recorded 85 days in Lalat to 94 days in IR87439 among Mid-Early duration varieties. Medium duration varieties showed flowering time from 95 days in IR88228 to 105 days in Swarna *Sub1* and Late duration varieties had flowering time variation from 96 days in Pratikshya to 110 days in IR85086 (Tables 1 and 2). The mean panicle length showed a minimum of 22.10 cm in Mahanadi with a standard error value of 0.25 to a maximum of 26.60 cm in IR87439 with a standard error value of 0.22. The flag leaf area varied from 25.62 cm^2 in Swarna to 56.08 cm^2 in IR85086. Fertility percentage showed a range from 79.36 % in Pratikshya to 93.93 % in Savitri *Sub1*. Moreover, fertility percentage ranged from 80.10 % (IR87439) to 88.06 % (Lalat) among Mid-Early duration varieties, 79.36% (Pratikshya) to 88.50 % (IR88228) among Medium duration varieties, and 85.60 % (IR85086) to 93.93 % (Savitri *Sub1*)among Late duration varieties. 100-grain weight varied significantly among the 10 varieties of rice. Swarna showed a minimum of 1.92 g and IR88228 showed a maximum of 3.79 g per 100-grain weight which indicates the size variation of the seeds among the varieties (Table 3). Correlation coefficient analysis revealed a significant correlation between plant height and flag leaf area (r=0.81) followed by 100 grain weight versus plant height (r=0.62), panicle length (r=0.56), flag leaf area (r=0.52), flag leaf area versus panicle length (r=0.48), duration of flowering (r=0.38) and panicle length versus plant height (r=0.38). The rest of the characters showed no significant or negative correlation among the agronomic traits (Table 3). ANOVA showed significant variations among all yield attributing characters (Table 4).

3.2 Estimation of genetic variation

Amplification with 31 RAPD primers out of the 60 tested primers generated 280 scorable DNA amplicons with a range size of 150 bp to 1960 bp (Table 5, Figs. 1a,b). The maximum mean polymorphic percentage obtained was 57.24%. A maximum 14 locus was amplified with the primer OPA04 followed by 13 loci in OPA14 and 12 loci in OPA15, OPB04, OPC01, and OPC08 (Table 5). The RAPD bands of highly significant varietal characteristics were observed in $OPA04_{750bp}$ in Swarna *Sub1*, Lalat and Swarna, whereas, $OPC08_{860bp}$ was found absent in Mahanadi. Swarna and Lalat showed a unique band of $OPC08_{700bp}$. IR87439, Swarna *Sub1*, Savitri *Sub1*, and Mahanadi produce $OPC08_{680bp}$ which was not found in Pratikshya and Lalat (Figs. 1a,b). No single primer was able to distinguish all the genotypes of rice. The polymorphic percentage varied from 19.36 % between IR8828 versus IR87439 to 45.59 % between Ciherang

Table 1

Different varieties of rice (*O. sativa*) with some important agronomic characters used for RAPD marker analysis.

Variety	Duration	Days to 50% flowering	Plant height (cm)±SE	Panicle length (cm)±SE	Flag leaf area (cm^2)±SE	Fertility % ±SE	100 grain wt. (g.) ±SE
IR87439	Mid-Early	93-94	104.33±0.69	26.60±0.22	33.88±0.07	80.10±0.76	2.48±0.022
Ciherang *Sub1*	Mid-Early	88-90	90.35±1.12	24.85±0.42	26.26±1.85	85.34±1.25	2.76±0.015
Lalat	Mid-Early	85-88	97.12±1.39	26.56±0.49	30.65±1.82	88.06±0.77	2.73±0.022
IR88228	Medium	95-96	110.15±0.80	26.55±0.47	42.56±0.90	88.50±0.29	3.79±0.029
Swarna *Sub1*	Medium	102-105	89.53±1.04	23.83±0.20	27.06±1.71	86.24±0.51	2.56±0.078
Pratikshya	Medium	96-98	91.70±1.15	25.30±0.23	28.88±1.56	79.36±0.35	2.22±0.07
IR85086	Late	108-110	120.25±1.98	26.48±0.14	56.08±2.53	85.60±0.96	2.77±0.055
Savitri *Sub1*	Late	100-102	105.83±1.78	23.09±5.14	30.86±1.28	93.93±0.45	2.18±0.50
Mahanadi	Late	104-106	102.60±1.29	22.10±0.25	38.70±2.93	88.41±0.71	2.15±0.016
Swarna	Late (Check variety)	101-103	89.95±0.75	22.70±0.59	25.62±0.099	87.92±2.67	1.92±0.052

Table 2

Variation of quantitative characters of 10 rice varieties of Odisha.

Characters	Range	Mean±SE	CV%
Plant height (cm)	87.73-115.93	100.13±2.80	08.85
Panicle length (cm)	22.09-27.40	24.69±0.57	07.35
Panicle number	09.32-18.10	13.67±0.82	19.02
Days to 50% flowering	89.00-105.00	98.13±2.16	06.98
Flag leaf length (cm)	23.98 – 45.54	32.19±1.82	17.95
Flag leaf area (cm^2)	22.54 – 56.13	32.51±2.96	28.88
Fertility %	78.98 – 93.08	86.57±1.07	03.92
100 grain weight	1.63 – 3.16	2.37±0.14	18.89

Table 3

Correlation coefficient of yield attributes of ten varieties of rice tested in the experiments.

	Duration to 50% flowering	Plant height	Panicle length	Flag leaf area	Fertility %
Plant height	0.226^{NS}				
Panicle length	-0.402^{*}	0.375^{*}			
Flag leaf area	0.380^{*}	0.812^{**}	0.480^{**}		
Fertility %	-0.012^{NS}	0.224^{NS}	-0.192^{NS}	-0.023^{NS}	
100 grain weight	-0.481^{**}	0.622^{**}	0.595^{**}	0.516^{**}	0.168^{NS}

NS=Not significant, *=Significant at ≥0.05, **=Significant at ≥0.01

Table 4

Analysis of variance (ANOVA) for different yield attributes of ten varieties of rice with *Sub1* gene introgression.

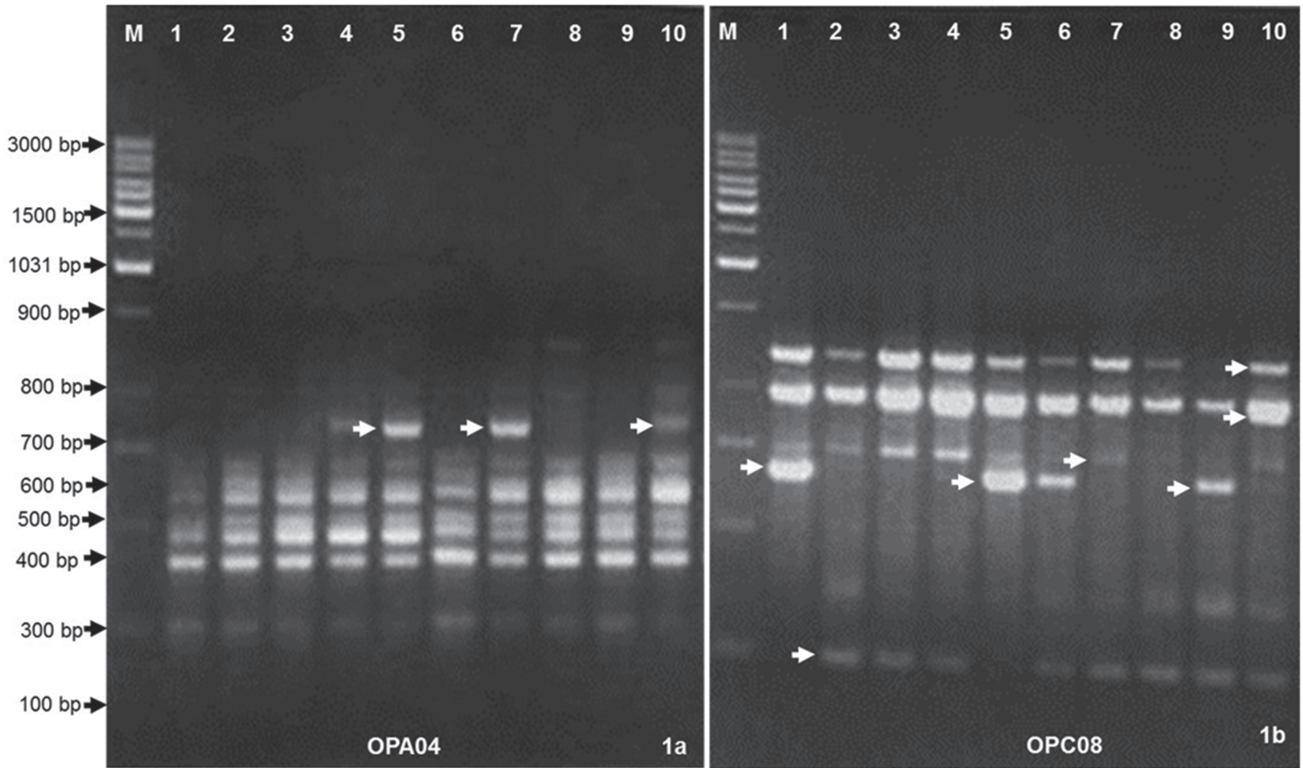
Source	DF	SS	MS	F
Plant height (cm)				
Between Groups	9	2362.981	262.553	26.846*
Within Groups	20	195.593	9.779	
Total	29	2558.574		
Panicle length (cm)				
Between Groups	9	99.137	11.015	24.494*
Within Groups	20	8.994	0.449	
Total	29	108.131		
Panicle number				
Between Groups	9	127.205	14.134	4.506*
Within Groups	20	62.726	3.136	
Total	29	189.932		
Flag leaf length (cm)				
Between Groups	9	1002.405	111.378	32.896*
Within Groups	20	67.713	3.385	
Total	29	1070.119		
Flag leaf area (cm^2)				
Between Groups	9	2645.289	293.932	33.006*
Within Groups	20	178.106	8.905	
Total	29	2823.495		
Fertile seed number				
Between Groups	9	263923.006	29324.778	25.076*
Within Groups	20	23387.993	2269.399	
Total	29	287310.994		
Fertility %				
Between Groups	9	346.455	38.495	6.726*
Within Groups	20	114.466	5.723	
Total	29	460.922		
100 seed weight (g)				
Between Groups	9	6.013	0.668	104.390*
Within Groups	20	0.128	0.006	
Total	29	6.141		

*Significant at ≥ 0.001 level. DF, degrees of freedom; SS, sum of squares; MS, mean squares; F,variance ratio.

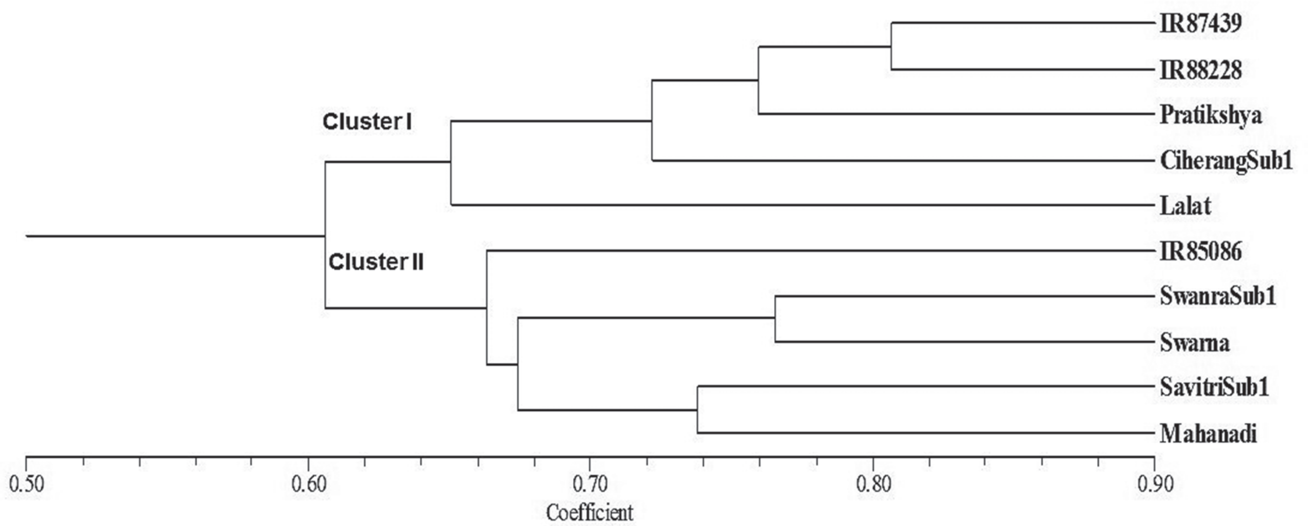
Table 5

Amplification pattern of RAPD bands of the 10 varieties of rice (*O. sativa*).

Sl. No.	Primer Name	Sequence 5'…..........3'	No. of allele	Number of Polymorphic bands	Number of monomorphic bands	Polymorphic %	Size range (bp)
1	OPA01	CAGGCCCTTC	8	5	3	62.50	300-1450
2	OPA02	TGCCGAGCTG	11	10	1	90.90	200-1200
3	OPA03	AGTCAGCCAC	8	5	3	62.50	280-1150
4	OPA04	AATCGGGCTG	14	8	6	57.14	150-1430
5	OPA05	AGGGGTCTTG	5	2	3	40.00	320-1180
6	OPA07	GAAACGGGTG	6	4	2	66.66	450-1450
7	OPA08	GTGACGTAGG	11	5	6	45.45	250-1600
8	OPA10	GTGATCGCAG	8	5	3	62.50	200-1180
9	OPA12	TCGGCGATAG	9	4	5	44.44	250-1250
10	OPA14	TCTGTGCTGG	13	8	5	61.53	150-1600
11	OPA15	TTCCGAACCC	12	5	7	41.66	250-1450
12	OPA17	GACCGCTTGT	9	4	5	44.44	200-1650
13	OPA19	CAAACGTCGG	11	4	7	36.36	300-1800
14	OPA20	GTTGCGATCC	8	6	2	75.00	240-900
15	OPB01	GTTTCGCTCC	9	7	2	77.77	350-1420
16	OPB03	CATCCCCCTG	5	2	3	40.00	245-1630
17	OPB04	GGACTGGAGT	12	8	4	66.66	190-1450
18	OPB06	TGCTCTGCCC	8	3	5	37.50	300-1650
19	OPB07	GGTGACGCAG	9	5	4	55.55	250-980
20	OPB08	GTCCACACGG	8	5	3	62.50	350-1650
21	OPB09	TGGGGGACTC	8	3	5	37.50	450-1480
22	OPB10	CTGCTGGGAC	9	5	4	55.55	200-1200
23	OPB11	GTAGACCCGT	9	6	3	66.66	180-1550
24	OPB12	CCTTGACGCA	8	6	2	75.00	250-1680
25	OPC01	CTCACCGTCC	12	8	4	66.66	200-1530
26	OPC03	GGGGGTCTTT	10	5	5	50.00	560-1700
27	OPC04	CCGCATCTAC	6	4	2	66.66	600-1875
28	OPC07	GTCCCGACGA	10	7	3	70.00	200-1220
29	OPC08	TGGACCGGTG	12	7	5	58.33	290-1680
30	OPC09	CTCACCGTCC	5	2	3	40.00	250-1960
31	OPC15	GACGGATCAG	7	4	3	57.14	155-900
		Total	280	162	118	1774.66	
		Mean	9.03	5.22	3.80	57.24	



Figs. 1. RAPD profiles of 10 varieties of rice amplified with different primers. (a)OPA04 (b) OPC08. M=DNA molecular weight marker, 1-10 lanes represent different variety of rice. 1=IR87439, 2=IR88228, 3=IR85086, 4=Ciherang *Sub1*, 5=Swarna *Sub1*, 6=Savitri *Sub1*, 7=Lalat, 8=Pratikshya, 9=Mahanadi, 10=Swarna.



Figs. 2. Dendrogram of 10 varieties of constructed using UPGMA based on Jaccard's correlation similarity coefficient of the RAPD characters.

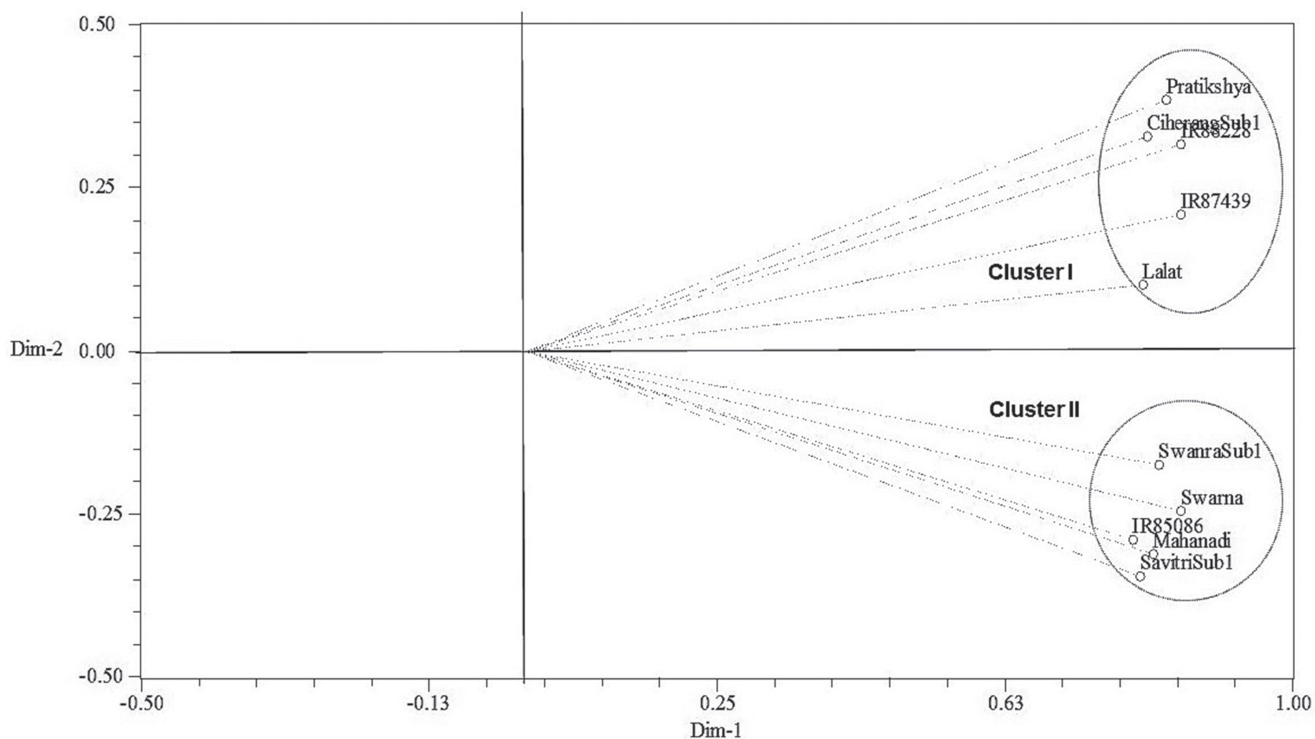


Fig. 3. Three-dimentional principal component analysis of 10 varieties of rice constructed using UPGMA based on Jaccard's similarity coefficients for the RAPD data set.

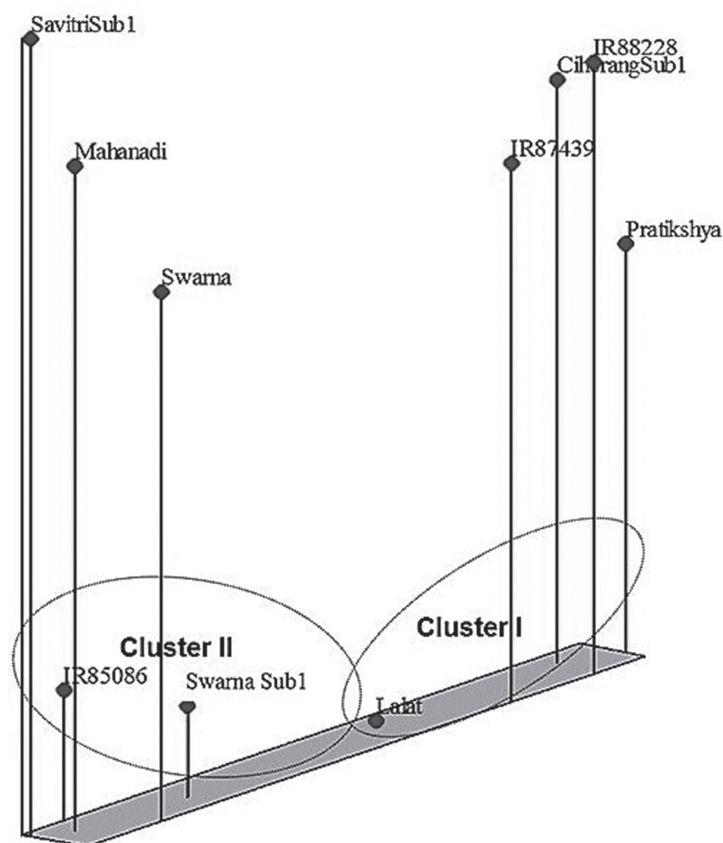


Fig. 4. Three-dimensional plot of principal component analysis of 10 varieties of rice using RAPD data showing genetic diversity.

Sub1 versus IR85086 which also showed a moderate genetic variation among these varieties at the DNA level (Table 5). The number of bands produced varied with each primer with an average mean of ~9.0 bands per primer.

3.3 Cluster analysis

Cluster analysis of RAPD bands showed two main clusters, i.e. Cluster-I having five Mid-Early and Medium duration varieties except for Swarna *Sub1* and Cluster-II having Late duration varieties with check variety Swarna (Fig. 2). Two-dimensional principal component analysis (PCA) showed also two clusters similar to cluster analysis dendrogram. The Cluster-II with Late duration varieties showed less genetic variability besides Swarna *Sub1* as a distinct variety in this group (Fig. 3). Cluster-I showed comparatively greater genetic variability where Lalat showed a distinct variation from the others. Three-dimensional PCA analysis clearly indicates that Lalat has higher genetic variability among all the studied varieties whereas the rest Mid-Early, Medium and Late duration varieties showed greater genetic variation between Cluster-I and Cluster-II (Fig. 4).

4. Discussion

4.1 Agronomic characteristics

The morphological variation was found significant among different characters as depicted in ANOVA (Table 4). The height of the plants showed significant variations along with leaf area and grain weight. Correlation coefficient analysis revealed a significant correlation between plant height and flag leaf area (r=0.81) followed by 100-grain weight versus plant height (r=0.62), panicle length (r=0.56), flag leaf area (r=0.52). Duration of flowering showed significant variation among the varieties. The maximum leaf area of 42.56 cm^2 was found in IR88228, a Medium duration variety, which also has a larger grain size having a maximum 100-grain weight of 3.79 g. The second highest leaf area 56.08 cm^2 was found in Late duration variety, IR85086, having 100 gain weight of 2.77 g. However, all the varieties showed more leaf area and 100-grain weight as compared to a check variety Swarna, having 25.62 cm^2 leaf area and 1.92g grain weight. Seed fertility had a maximum of 93.93 % in Savitri *Sub1* followed by 86.24 % in Swarna *Sub1* and 85.34 % in Ciherang *Sub1*. Thus, introgression of submergence gene (*Sub1*) into these varieties has not much effect on seed fertility percentage. The morphological variation of rice agronomic characters was also reported earlier in different landraces of India (Dikshit *et al.*, 2013). Although, considerable crop genetic diversity continues to be maintained in farms in traditional varieties (Jarvis *et al.*, 2008). The high dominance occurred of all the valuable alleles, with low frequency of the variety richness suggested that diversity may be maintained as insurance to meet future environmental changes. The proportion of genetic diversity in autogamous species, such as rice, is expected to be greater amongst those within each landrace.

4.2 Phylogenetic and PCA analysis

Dendrogram constituted with RAPD markers showed a single common ancestry that basically formed Cluster-I (five Mid-Early and Medium duration varieties including Ciherang *Sub1*) and -II (four Late duration varieties including check variety Swarna and a Medium duration variety Swarna *Sub1*). Principal component analysis (PCA) in 2-D form could distinguish four varieties (IR87239, IR88228, Pratikshya, Ciherang *Sub1*) forming minor clusters from Lalat with a separate branch forming Cluster-I. Similarly, among the Late duration varieties, IR85086 formed a separate branch while Swarna and Swarna *Sub1* formed a single minor cluster showing their genetic affinity and Savitri *Sub1* and Mahanadi formed a single minor cluster with little higher genetic variability forming Cluster-II (Fig. 3). Similar types of RAPD derived clustering were also reported in ten Indian rice varieties by Karande *et al.,* (2017). The average sizes of amplicons were between 150 bp to 1900 bp which is in accordance with the other reports of traditional lowland rice varieties of Assam (Bhuyan *et al.*, 2007), Pakistan (Ukoskit, 2004), wild rice of Thailand (Arif *et al.*, 2005). It is expected that the same length of DNA fragments is from the appropriate locus, and represent the dominant single locus with two possible alleles (Nagy *et al.*, 2012) in RAPD markers. The RAPD profiles in the present study displayed a moderate degree of polymorphism which confirms the suitability of RAPD markers for discrimination of different varieties of rice plants. Our study yielded highly reproducible RAPD fingerprints which might be a useful tool for discrimination of genetic variation in ten varieties of rice specially submergence introgression varieties in the normal environment. A high level of genetic diversity among the non-basmati groups holds a promise in the quest of conserving crop diversity and broadening the gene pool for breeding (Mathure *et al.*, 2010). The average polymorphism of 57.24 % in our study found the contrast to a high polymorphic percentage (80 to 95%) in rice genotypes (Yu and Nguyen, 1994; Raghunathachari *et al.*, 2000; Davierwala *et al.*, 2000; Ravi *et al.*, 2003; Rabbani *et al.*, 2008; Ray *et al.*, 2012; Roopa and Chikkaswamy, 2016; Tahmina *et al.*, 2017. However, our result could be in accordance with the result of Beverley *et al.,* 1997; Choudhury *et al.,* 2001 and Kanawapee *et al.*, 2011 having 50% to 68.94% polymorphism in rice using RAPD markers. In contrast, hybrid rice parental lines of rice in Iran revealed only 35% polymorphism using 15 RAPD primers (Kiani and Katalani, 2018). However, very low polymorphism was recorded in PCA clearly distinguished all the clusters of Mid-Early and Medium

duration varieties and Late duration varieties. It is evident that Lalat, a Mid-Early duration variety, of Cluster-I found distinct forming a separate branch showing high genetic variability as compared to the rest of the varieties. Cluster-II with all the Late duration varieties found a high variation of genetic makeup with IR85086 having distinct genetic distance from the rest of the varieties. It was evident from the fact that rice varieties belonging to different maturity time were grouped into the same phylogenetic cluster. Partially salt-tolerant local and inbred rice of Bangladesh was reported to distinguish with the use of Operon primer using RAPD (Mazumder *et al.*, 2020).

The dendrogram analysis provides clusters on the basis of similarity which has high efficiency and the ability for variety identification (Aliyu *et al.*, 2000). Overall, this study reveals that the rice germplasms of Odisha have moderate genetic diversity. Nowadays simple sequence repetitive DNA markers (SSR) are used for genetic diversity evaluation of rice rather than RAPD markers. A variety of studies with SSR markers have been reported to distinguish rice varieties which could be useful for co-dominant maker analysis (Shahriar *et al.*, 2014; Shakil *et al.*, 2015; Siddique *et al.*, 2016; Rashid *et al.*, 2018; Syed *et al.*, 2019; Verma *et al.*, 2019) as compared to dominant alleles in RAPD marker. Thus, SSR marker analysis in different duration of rice variety could be more useful for genetic variation study in the future among rice germplasms of Odisha.

5. Conclusion

The present study resulted in the development of RAPD markers that can be efficiently used for the genetic diversity assessment of different varieties of rice. On the basis of morphological traits and RAPD markers, Mid-Early and Medium duration varieties of Odisha formed separate clusters (IR87439, Ciherang *Sub1*, Lalat, and IR88228) with the highest grain weight in IR88228 in Odisha condition compared to check varieties. Thus, the putative yield attributing polymorphic RAPD loci found in this study can be used in mapping and linkage analysis. The polymorphic markers could be potential for developing a decision support system for creating crosses based on RAPD-based genetic distance matrices. The introgression of the *Sub1* gene has not much effect on fertility % and grain weight attributing characters. The genetic diversity knowledge could be a useful tool for the identification of duplicates in maintaining genetic stock to make a core collection of pre-breeding genotypes. The assessment of genotypes based on yield attributing phenotypic characters along with RAPD alleles seems to be a more reliable strategy for the selection of parents in hybridization.

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Conflict of interest

All author declares any conflict of interest in the paper.

References

Alam, M.S., Begum, S.N., Gupta, R., Islam, S.N. (2014). Genetic diversity analysis of rice (*Oryza sativa* L.). Int. J. Agril. Res. Innov. & Tech. 4 (1): 77-87

Aliyu, B., Ng, N.Q., Fawole, I. (2000). Inheritance of pubescences in crosses between cowpea (*Vigna unquiculata* (L)) Walp. and *V. rhomboidea* Burtt. Davy. Niger J Genet. 15 (1): 9–14.

Anonymous (2020). World rice acreage 2010-2019. Statista Research Department. https://www.statista.com/statistics/271969/world-rice-acreage-since-2008/

Arif, M., Kousar, S., Bajwa, A., Arif, A., Zafar, Y. (2005). Genetic diversity among rice genotypes of Pakistan through random amplified polymorphic DNA (RAPD) analysis. Pakistan Journal of Botany 37: 585-592.

Arshad, S., Iqbal, A., Nawaz, S., Ahmed, N. (2011). Genetic diversity studies of coarse and fine rice using RAPD markers. Front. Agric. China 5(2): 129–134.

Baishya, S., Sachdev, A., Johari, R. P., Mehta, S. L. (2000). RAPD analysis of aromatic and non-aromatic rice *(Oryza sativa* L.). J. Plant Biochemistry & Biotechnology 9: 23-26.

Beverley, J.P., Newbury, H.J., Jackson, M.T., Ford-Lloyd, B.V. (1997). Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Mol. Breeding 3:115-125.

Bhuyan, N., Borah, B.K., Sarma, R.N. (2007). Genetic diversity analysis in traditional lowland rice (*Oryza sativa* L.) of Assam using RAPD and ISSR markers. Current Science 93: 967-972.

Bowditch, B.M., Albright, D.G., Williams, J.G.K., Braun, M.J. (1994). Use of randomly amplified polymorphic DNA markers in comparative genome studies. Methods Enzymol 224:294-309.

Choudhury, P.R., Kohli, S., Srinivasan, K., Mohapatra, T., Sharma, R.P. (2001). Identification and classification of aromatic rice based on DNA fingerprinting. Euphytica 118**:** 243-251.

Dally, A. M., Second, G. (1990). Chloroplast DNA diversity in wild and cultivated species of rice (Genus *Oryza,*

Section Oryza) – Cladistic-mutation and genetic-distance analysis. Theor. Appl. Genet. 80:209-222.

Das, S. R. (2013). Reflections on >40 years of rice breeding for eastern India. SABRAO Journal of Breeding and Genetics 45(1):21-26.

Das, S.R. (2018). Conventional breeding approaches for enhancing yield potential of rice. Oryza 55 (Spl. Issue): 1-17.

Davierwala, A.P., Chowdari, K.V., Kumar, S., Reddy, A.P., Ranjekar, P.K., Gupta, V.S. (2000). Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. Genetica 108 (3): 269–284.

Dikshit, N., Das, A. B., Sivaraj, N., Kar, M.K. (2013). Phenotypic diversity for agro-morphological traits in 105 landraces of rice (*Oryza sativa* L.) from Santhal Parganas, Jharkhand, India. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. 83(3):291-304.

Fukuoka, S., Hosaka, K., Kamijima, O. (1992). Use of random amplified polymorphic DNAs (RAPDs) for identification of rice accessions. Japan. J. Genet. 67:243-252.

Hadrys, H., Balick, M., Schierwater, B. (1992). Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol Ecol. 1: 55-63.

Hasan, M., Raihan, M. S. (2015). Genetic variability in Bangladeshi aromatic rice through RAPD analysis. Turkish Journal of Agriculture - Food Science and Technology 3(3): 107-111,

Ishii, T., Terachi, T., Mori, N., Tsunewaki, K. (1993). Comparative study on the chloroplast, mitochondrial and nuclear genome differentiation in 2 cultivated rice species, *Oryza sativa* and *O. glaberrima*, by RFLP analysis. Theor. Appl. Genet. 86:88-96.

Jaccard, P. (1908). Nouvellesrecherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. XLIV 163: 223-269.

Jarvis, D. I., Brown, A. H. D., Cuong, P. H., Collado-Panduro, L., Latournerie-Moreno, L., Gyawali, S., Tanto, T., Sawadogo, M., Mar, I., Sadiki, M., Hue, N. T., Arias-Reyes, L., Balma, D., Bajracharya, J., Castillo, F., Rijal, D., Belqadi, L., Rana, R., Saidi, S., Ouedraogo, J., Zangre, R., Rhrib, K., Chavez, J. L., Schoen, D., Sthapit, B., Santis, P. D., Fadda, C., Hodgkin, T. (2008). A global perspective of the richness and evenness of traditional crop-variety diversity maintained by farming communities. Proc Natl Acad Sci USA 105:5326–5331.

Joshi, B.K., Bimb, H.P., Kansakar, D., Ghimire, E. (2012). Genetic relationship among Nepalese rice landraces and cultivars based on RAPD markers. Nepal Journal of Biotechnology 2(1):16-25.

Kanawapee, N., Sanitchon, J., Srihaban, P., Theerakulpisut, P. (2011) Nov. Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. Electron. J. Biotechnol. 14 (6): 2-2.

Karande, P.T., Nandeshwar, B.C., Kokane, A.D., Chavhan, R.L., Dethe, A.M. (2017). Assessment of genetic diversity using RAPD marker among different varieties of rice (*Oryza sativa*). International Journal of Tropical Agriculture 35 (3): 509-516.

Kiani, G., Katalani, C. (2018). Divergence in hybrid rice parental lines detected by RAPD and ISSR markers. Acta Agric. Slov. 111 (2): 369–376.

Mackill, D.J. (1995). Plant genetic resources: classifying Japonica rice cultivars with RAPD markers. Crop Sci. 35: 889-894.

Mathure, S., Altafhusain, N., Narendra, J. (2010). Diversity analysis in selected non-basmati scented rice collection (*Oryza sativa* L.). Rice Science 17: 35–42.

Mazumder, S.R., Hoque, H., Sinha, B., Chowdhury, W. R., Hasan, Md N., Prodhan, S.H. (1999). Genetic variability analysis of partially salt tolerant local and inbred rice (*Oryza sativa* L.) through molecular markers. Heredity 82: 638-644.

Mazumder, S.R., Hoque, H., Sinha, B., Chowdhury, W.R., Hasan, N. M., Prodhan, S.H. (2020). Genetic variability analysis of partially salt tolerant local and inbred rice (*Oryza sativa* L.) through molecular markers. Heliyon 6: e04333. https://doi.org/10.1016/j.heliyon.2020.e04333

Nagy, S., Poczai, P., Cernák, I., Gorji, A.M., Hegedus, G., Taller, J. (2012). PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. Biochem. Genet. 50: 670-672.

Nakano, M., Yoshimura, A., Iwata, N. (1992). Phylogenetic study of cultivated rice and its wild relatives by RFLP. Rice Genet. Newsl. 9: 132-134.

Rabbani, M.A., Pervaiz, Z.H., Masood, M.S. (2008). Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. Electron. J. Biotechnol. 11 (3): 52–61.

Raghunathachari, P., Khanna, V. K., Singh, U. S., Singh, N. K. (2000). RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.). Curr Sci. 79: 994-998.

Rahman, S.N., Islam, M.S., Alam, M.S., Nasiruddin, K.M. (2007). Genetic polymorphism in rice (*Oryza sativa* L.) through RAPD analysis. Indian Journal of Biotechnology. 6: 224-229.

Rajani, J., Deepu, V., Nair, G. M., Nair, A. J. (2013). Molecular characterization of selected cultivars of rice, *Oryza sativa* L. using Random Amplified Polymorphic DNA (RAPD) markers.International Food Research Journal 20(2): 919-923.

Rashid, M.M., Shahin, I., Islam, M.A., Lutful, H. (2018). Genetic diversity analysis of rice landraces (*Oryza*

sativa L.) for salt tolerance using SSR markers in Bangladesh. Fundamental and Applied Agriculture 3 (2): 460–466.

Ravi, M., Geethanjali, S., Sameeyafarheen, F., Maheswaran, M. (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD. Euphytica 133: 243–252.

Ray, A., Pattanaik, A., Samal, K.C., Kshirsagar, S.S., Rout, G.R. (2012). Variety diagnostic PCR-RAPD markers for aromatic rice varieties grown in Eastern Part of India. J. Plant Sci. Res. 28: 91-100.

Rohlf, F. J. (2008). NTSYSpc: Numerical taxonomy system, ver.2.20. Exeter Publishing Ltd., Setauket, NY, USA.

Roopa, B.R., Chikkaswamy, B.K. (2016). An investigation of RAPD analysis of genetic variability of some rice germplasm (*Oryza sativa* L.). Int. J. Curr. Microbiol. App. Sci. 5 (9): 843–849.

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, H. A., Allard, H. A. (1984). Ribosomal DNA spacer length polymorphism in barley. Mendelian inheritance, chromosomal locations and population dynamics. Proc. Nat. Acad. Sci. USA 81: 8014-8018.

Shahriar, M.H., Robin, A.H., Begum, S.N., Hoque, A. (2014). Diversity analysis of some selected rice genotypes through SSR-based molecular markers. J. Bangladesh Agric. Univ. 12 (2): 307–311.

Shakil, S.K., Sultana, S., Hasan, M.M., Hossain, M.M., Ali, M.S., Prodhan, S.H. (2015). SSR marker based genetic diversity analysis of modern rice varieties and coastal landraces in Bangladesh. Indian J. Biotechnol. 14: 33–41.

Shaptadvipa, B., Sarma, R.N. (2009). Assessment of natural magnitude of genetic diversity based on DNA polymorphism with RAPD technique in traditional glutinous rice (*Oryza sativa* L.) of Assam. Asian Journal of Plant Sciences. 8(3):218-223.

Siddique, M.A., Khalequzzaman, M., Fatema, K., Islam, M.Z., Islam, M.M., Chowdhury, M.A. (2016). Molecular characterization and genetic diversity of Aman rice (*Oryza sativa* L.) landraces in Bangladesh. Bangladesh Rice J. 20 (2): 1-1.

Singh, A., Sengar, R. S. (2015). DNA fingerprinting based decoding of indica rice (*Oryza sativa* L) via molecular marker (SSR, ISSR, & RAPD) in Aerobic condition. Adv Crop Sci Tech 3: 167. doi:10.4172/2329-8863.1000167

Syed, M.A., Iftehkaruddaula, K.M., Akter, N., Biswas, P.S. (2019). Molecular diversity analysis of some selected BBRI released rice varieties using SSR markers. Int. Res. J. Biol. Sci. 1 (2): 51–58.

Tahmina, I., Rahman, M.I.S., Hoqueand, R.H., Sarker (2017). Genetic diversity assessment in ten aromatic rice varieties of Bangladesh. Plant Tiss. Cul. Biotechnol. 27: 217-225.

Ukoskit, K. (2004). Genetic diversity of wild rice (*Oryza* spp.) in the northern region of Thailand. Thammasat International Journal of Science and Technology. 9: 11-20.

Verma, H., Borah, J.L., Sarma, R.N. (2019). Variability assessment for root and drought tolerance traits and genetic diversity analysis of rice germplasm using SSR markers. Sci. Rep. 9 (1): 1–9.

Welsh, J., McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.

Williams, J. G. K., Kubelik, A.R., Livak, K. J., Rafalski, J. A., Tingey, S.V. (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

Yu, L.X., Nguyen, H. T. (1994). Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theor. Appl. Genet. 87:668-672.

Zakiyah, N.M., Handoyo, T., Kim, K.-Min. (2019). Genetic diversity analysis of Indonesian aromatic rice varieties (*Oryza sativa* L.) using RAPD. J. Crop Sci. Biotech. 22 (1): 55-63.

Zhang, Q.F., Maroof, M. A.S., Lu, T.Y., Shen, B. Z. (1992). Genetic diversity and differentiation of indica and japonica rice detected by RFLP analysis. Theor. Appl. Genet. 83:495-499.

Zheng, K. L., Shen, B., Quian, H. R. (1991). DNA polymorphisms generated by arbitrary primed PCR in rice. Rice Genet. Newsl. 8:134-136.