International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 11, Issue 13, 2019, pp.-8713-8718.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

MOLECULAR DIVERSITY ANALYSIS OF SOME AROMATIC RICE (*ORYZA SATIVA* L.) GENOTYPES USING SSR MARKERS

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Received: June 30, 2019; Revised: July 11, 2019; Accepted: July 12, 2019; Published: July 15, 2019

Abstract: Rice is one of the world's most important cereal crops. It belongs to grass family Poaceae (2n = 24). There are 25 species of genus Oryza, and only two species, namely *Oryza sativa* and *Oryza glaberrima* are cultivated. Aromatic rice constitutes a small but an important sub-group of rice. The genetic diversity study was done using 24 SSR markers out of which 11 were polymorphic. The dendrogram generated by using Darwin 6.0.15 software identified three major clusters (I, II, and III). Cluster I is the largest cluster with 15 genotypes, Cluster II is the second largest cluster with 13 genotypes and Cluster III consisted of 4 genotypes. Among the polymorphic markers, number of alleles ranged from 2 (RM11, RM25 and RM 552), 3 (RM125), 4 (RM44, RM316, RM215 and RM271) and 6 (RM447). Highest PIC value was recorded for RM447 (0.750) and lowest for RM125 (0.236). Heterozygosity (He) value ranged from 0.127 (RM484) to 0.782 (RM447). Maximum diversity was observed between IC-137401 and IC-342368 (0.98), followed by IC-342368 and IC-326284 (0.97). The genetic diversity studies conducted will help in further crop improvement programmes in identification and germplasm preservation.

Keywords: *Aromatic Rice, SSR, Genetic Diversity, Cluster Analysis, PIC*

Citation: Rani W., *et al*., (2019) Molecular Diversity Analysis of Some Aromatic Rice (*Oryza sativa* L.) Genotypes Using SSR Markers. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 11, Issue 13, pp.- 8713-8718.

Introduction

Rice (*Oryza sativa* L.) (2n = 24) belonging to the family Poaceae is the staple food for half of the world's population and occupies almost one-fifth of the total land area covered under cereals. Rice is bestowed with great genetic diversity accounting for more than 100,000 landraces and improved cultivars. Rice is the most diversified crop species due to its adaption to a wide range of geographical, ecological and climatic regions [1]. The quality preferences of rice consumers have also resulted in a wide diversity of varieties specific to different localities. Although the exact diversity cannot be gauged, it is estimated to be around 140000 different genotypes. The IRRI gene bank preserves nearly 100000 accessions. India alone has about 86330 accessions, of which 42004 are in the national gene bank [2], which is enriched by further explorations, collections and conservation. The assessment of genetic diversity is important in rice improvement activity in terms of selection, conservation and meaningful utilization. It was well understood about the role of broad genetic base and properly characterized germplasm in the crop improvement of cultivated plants. To fulfil the need of continuous demand of varietal improvement, evaluation and characterization of existing germplasm is required. Selection of suitable parent for hybridization in crop improvement, study related to genetic variability assessment of germplasm including landraces, advanced breeding lines and cultivated varieties of the concerned crop is necessary as sound breeding depends on the extent of variability in the base population. Genetic diversity is normally measured by either genetic dissimilarity or genetic similarity, which imply either differences or similarity at the genetic level [3]. Characterization of genotypes/varieties by using morphological markers and molecular markers will be quite satisfactory because of less number of stack morphological markers as compared to DNA based markers. The DNA based markers are the most effective and promising tools nowadays for varietal identification and characterization as such markers are more reliable and remain unaffected by change in external environment like crop season, temperature, rainfall and agronomic practices. Amongst the polymerase chain reaction (PCR) based markers, the microsatellites also known as simple sequence repeats (SSRs) are useful as genetic markers because they detect high levels of allelic diversity. These are reproducible, co-dominant and distributed throughout the genome. The advent of genomic sequences in rice offers new opportunities to enhance the density of locus specific and polymorphic markers for high-resolution genetic analysis. Owing to technical efficiency and multiplex potential, these markers are preferable for many forms of high throughput mapping, genetic analysis and marker assisted plant improvement strategies [4].

Materials and Methods

The experimental materials comprised of 32 rice genotypes including three checks *viz*., Bahadur (non-aromatic), Ja-Pnah (aromatic) and Chakhao Poireiton (aromatic) which were collected from ICAR-NBPGR Shillong, Manipur, Meghalaya and Mizoram. The materials were grown in completely randomized block design in three replications during *kharif* 2017 carried out at Research cum Instructional Farm, College of Post Graduate Studies in Agricultural Sciences, , Umiam, 793103. Leaf samples of 20 - 25 days old were collected during morning hours and stored at - 20ºC for DNA extraction and further use.

Isolation of Genomic DNA

Genomic DNA from leaf samples were isolated using CTAB extraction method according to [5] with few modifications in composition of DNA extraction buffer. The method is briefly described as follow; the material was sterilized with 70% ethanol. Leaf tissue (20 - 25 days old) was grinded in mortar and pestle with 1 ml buffer and 1 ml of washing (2 ml total). The paste was then transferred in tube (2 ml micro centrifuge tube). The tubes were then incubated at 65ºC for 20 - 30 minutes (shaken every 5 minutes), then centrifuged for 5 minutes at 5000 rpm; the debris was then removed. 800 µl of supernatant was taken in another tube and equal amount of chloroform: isoamyl alcohol (24:1) *i.e.* 800 µl was added.

Table-1 *List of SSR primers used in the present study*

S	Chromosome Number	Marker	Forward sequence	Reverse sequence
1	7	RM125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
2	7	RM11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
3	7	RM455	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
4	7	RM118	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG
5	8	RM408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
6	8	RM152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG
7	8	RM25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC
8	8	RM44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
9	8	RM284	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC
10	8	RM433	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC
11	8	RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
12	9	RM316	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
13	9	RM105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
14	9	RM215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
15	10	RM474	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG
16	10	RM271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC
17	10	RM171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG
18	10	RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
19	11	RM552	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC
20	11	RM536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC
21	11	RM287	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
22	11	RM144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG
23	12	RM19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
24	12	RM277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG

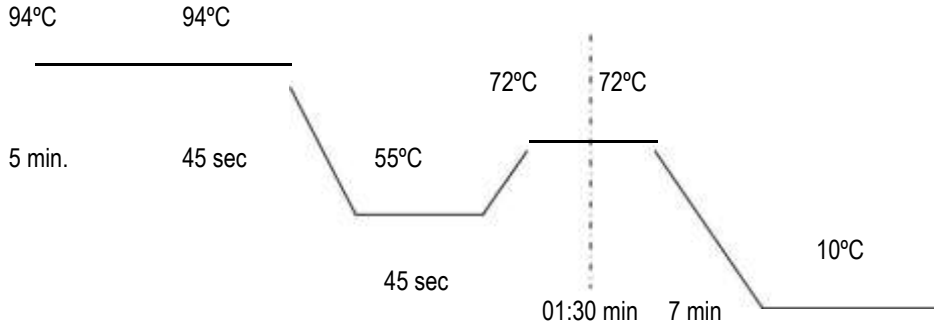


Fig-1 PCR Setting

Table- 3 *Details of polymorphic SSR markers*

Markers	Chromosome number	Range of fragments (bp)	TNA	TNP	Percent polymorphism	Number of alleles per locus	PIC	He
RM125	7	90-150	34	5	14.71	3	0.236	0.258
RM11	7	120-150	25	7	28.00	2	0.322	0.403
RM25	8	120-165	48	24	50.00	2	0.375	0.500
RM44	8	80-135	28	8	28.57	4	0.412	0.452
RM447	8	95-155	36	25	69.44	6	0.750	0.782
RM316	9	120-225	29	17	58.62	4	0.585	0.654
RM215	9	125-160	27	11	40.74	4	0.527	0.579
RM271	10	70-120	28	13	46.43	4	0.501	0.579
RM484	10	285-305	30	2	6.67	3	0.123	0.127
RM552	11	155-260	27	5	18.52	2	0.256	0.302
RM277	12	110-125	30	18	60.00	4	0.618	0.678
Total			342	135	421.7			
Average			31.09	12.27	38.34	3.45	0.428	

TNA=Total number of amplified alleles, TNP=Total number of polymorphic alleles

After centrifugation at 13000 rpm for 15 minutes, the aqueous phase was taken in another tube (450 µl). 2/3rd volume of isopropanol was added and mixed thoroughly by inverting and allow DNA to precipitate for 15 - 20 minutes. Again after centrifugation at 13000 rpm for 15 minutes, the pellet was washed with 70% ethanol (500µl) and again centrifuged at 10000 rpm for 10 minutes and dried in tissue paper for 1 - 2 hour and stored in TE (30 µl) at - 20ºC or 4ºC.

CTAB Buffer Composition (10 ml)

100 mM Tris HCl (pH 8.0)	1.0 ml
20 mM EDTA (pH 8.0)	0.5 ml
5 M NaCl	3.0 ml
2% CTAB (w/v)	1.0 ml
Sterile distilled water	4.5 ml

DNA Quantification and Dilution

DNA quantification can be done by Gel Electrophoresis method. The extracted genomic DNA quantity was checked on 0.8% agarose gel.

Genomic DNA Amplification in Polymerase Chain Reaction (PCR)

The genomic DNA was subjected to PCR amplification as per the procedure described by [6] with minor modifications. The PCR was carried out using a programmable thermo cycler for DNA amplification.

Agarose Gel Electrophoresis

The PCR products were resolved in 2% Agarose gel prepared in 0.5X TBE buffer stained with Ethidium Bromide.

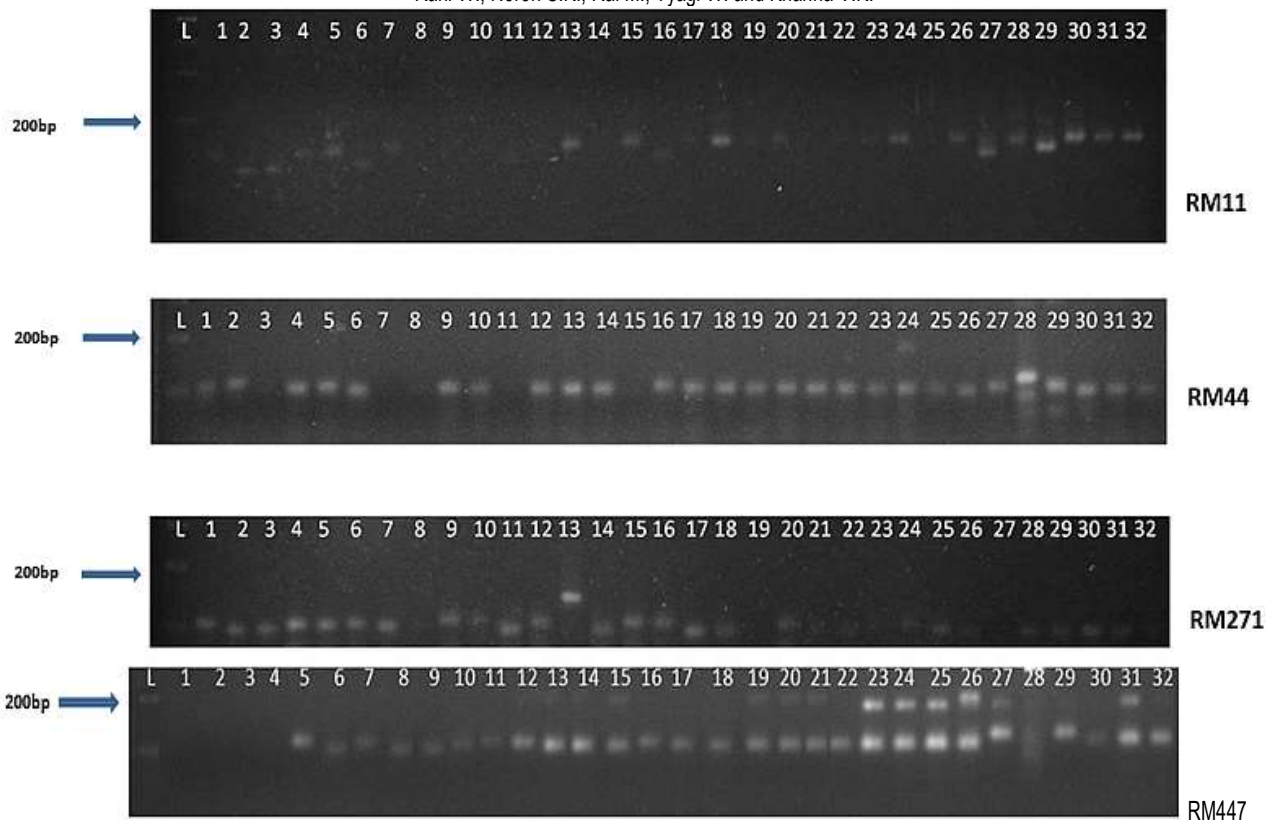


Fig-2 Amplification pattern of some polymorphic SSR markers of 32 rice genotypes, [1-32 – Rice genotypes]

Visualization of bands

After electrophoresis, the products were visualized under UV transilluminator and recorded under GEL-DOC system.

Detection of varietal polymorphism using simple sequence repeats (SSR) primers.

The varietal polymorphism was detected by using 24 SSR primers. The primers used for this purpose are presented in [Table-1].

Data Analysis

For 32 genotypes under study, the molecular weight of DNA was visually estimated from the obtained bands by comparing with 100 bp gene ruler.

Scoring of SSR Bands

Amplified products from SSR markers analysis were scored qualitatively for the presence or absence of the corresponding band among the genotypes. Only the clear and unambiguous amplified bands were scored. The presence or absence of each band in all genotypes was scored manually with '1' indicating the presence of the band and '0' indicating the absence of the band. To measure the informativeness of the markers, the polymorphism information content (PIC) for each SSR markers was calculated from online sources.

Clustering pattern by using dendrogram

Unweighted Neighbor-Joining clustering was performed on Simple Matching Coefficient and Pairwise Dissimilarity Matrix utilizing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method. Data analysis was done using the software Darwin 6.0.15.

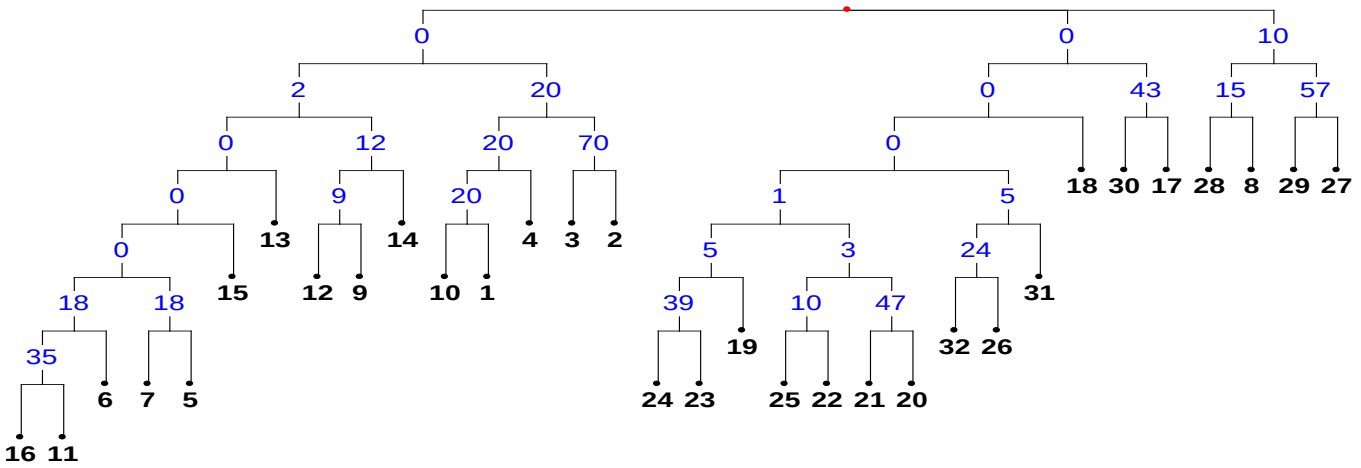
Results and Discussion

In this present study, molecular diversity in aromatic rice was done by using SSR markers. A total of 24 SSR markers were used to screen diversity in 32 rice genotypes [Table-2]. Out of the total primers used, 11 markers were found to be polymorphic and also in the remaining 13 primers, amplification was not observed with some primers, some being monomorphic and some having vague bands and unclear fragments.

Table-2 *Aromatic rice genotypes along with check varieties*

S	Genotype code	Genotypes	Source
1	R1	IC-89071	ICAR-NBPGR, Shillong
2	R2	IC-137342	-do-
3	R3	IC-137401	-do-
4	R4	IC-323776	-do-
5	R5	IC-323781	-do-
6	R6	IC-326284	-do-
7	R7	IC-342353	-do-
8	R8	IC-342368	-do-
9	R9	IC-342369	-do-
10	R10	IC-351517	-do-
11	R11	IC-394788	-do-
12	R12	IC-464363	-do-
13	R13	IC-464661	-do-
14	R14	IC-464684	-do-
15	R15	IC-465275	-do-
16	R16	Tai Sanghan	Mizoram
17	R17	CT3-D-1	Manipur
18	R18	CT3-D-2	-do-
19	R19	CT3-D-4	-do-
20	R20	CT3-D-6	-do-
21	R21	CT3-D-7	-do-
22	R22	CT3-D-9	-do-
23	R23	CT3-D-10	-do-
24	R24	CT3-D-11	-do-
25	R25	CT3-D-12	-do-
26	R26	Mantup Chakhao	-do-
27	R27	Kaunglauny Chakhao	-do-
28	R28	Faisenbuman Chakhao	-do-
29	R29	Bahadur	Meghalaya
30	R30	Ja-Pnah	-do-
31	R31	Chakhao Poireiton	Manipur
32	R32	Chakhao Amubi	-do-

The results indicated a high level of genetic variation in the germplasm tested. The polymorphic markers used in this study showed distinct polymorphism among the rice genotypes screened indicating the robustness of the markers used. A total of 38 different reproducible bands (alleles) ranging in size from 70 bp to 305 bp were amplified [Fig-2].



1 - IC-89071, 2 - IC-137342, 3 - IC-137401, 4 - IC-323776, 5 - IC-323781, 6 - IC-326284, 7 - IC-342353, 8 - IC-342368, 9 - IC-342369, 10 - IC-351517, 11 - IC-394788, 12 - IC-464363, 13 - IC-464661, 14 - IC-464684, 15 - IC-465275, 16 -Tai Sanghan, 17 - CT3-D-1, 18 - CT3-D-2, 19 - CT3-D-4, 20 - CT3-D-6, 21 - CT3-D-7, 22 - CT3-D-9, 23 - CT3-D-10, 24 - CT3-D-11, 25 - CT3-D-12, 26 - Mantup Chakhao, 27 - Kaunglauny Chakhao, 28 - Faisenbuman Chakhao, 29 - Bahadur, 30 - Ja-Pnah, 31 - Chakhao Poireiton, 32 - Chakhao Amubi.

Fig-2 Unweighted Neighbor-Joining Tree (Hierarchical view) between thirty two rice genotypes generated from 11 polymorphic SSR markers

	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30	R31
R1																															
R2	0.53																														
R3	0.61	0.27																													
R4	0.29	0.45	0.54																												
R5	0.76	0.77	0.85	0.69																											
R6	0.77	0.78	0.86	0.7	0.74																										
R7	0.67	0.68	0.76	0.6	0.45	0.65																									
R8	0.89	0.9	0.98	0.81	0.95	0.97	0.86																								
R9	0.64	0.65	0.73	0.57	0.67	0.68	0.58	0.83																							
R10	0.36	0.58	0.66	0.34	0.81	0.82	0.72	0.94	0.69																						
R11	0.61	0.62	0.7	0.54	0.58	0.49	0.49	0.81	0.52	0.66																					
R12	0.61	0.62	0.7	0.54	0.64	0.65	0.55	0.81	0.36	0.66	0.49																				
R13	0.57	0.57	0.66	0.49	0.56	0.57	0.47	0.76	0.47	0.61	0.41	0.44																			
R14	0.54	0.55	0.63	0.46	0.57	0.58	0.48	0.73	0.33	0.59	0.42	0.3	0.37																		
R15	0.58	0.58	0.67	0.5	0.54	0.56	0.45	0.77	0.48	0.62	0.4	0.46	0.37	0.38																	
R16	0.72	0.73	0.81	0.65	0.69	0.6	0.59	0.91	0.63	0.77	0.36	0.6	0.52	0.53	0.5																
R17	0.59	0.6	0.68	0.51	0.65	0.67	0.56	0.74	0.53	0.64	0.51	0.51	0.46	0.43	0.47	0.61															
R18	0.67	0.68	0.76	0.6	0.74	0.75	0.65	0.83	0.62	0.72	0.59	0.59	0.54	0.52	0.55	0.7	0.46														
R19	0.6	0.61	0.69	0.53	0.67	0.68	0.58	0.76	0.55	0.65	0.52	0.52	0.47	0.45	0.48	0.63	0.39	0.44													
R20	0.64	0.64	0.73	0.56	0.7	0.72	0.61	0.79	0.58	0.68	0.55	0.56	0.51	0.48	0.52	0.66	0.42	0.47	0.33												
R21	0.65	0.66	0.74	0.58	0.72	0.73	0.63	0.81	0.6	0.7	0.57	0.57	0.53	0.5	0.54	0.68	0.44	0.49	0.35	0.18											
R22	0.59	0.6	0.68	0.52	0.66	0.67	0.57	0.75	0.54	0.64	0.51	0.51	0.46	0.44	0.47	0.62	0.38	0.43	0.28	0.28	0.3										
R23	0.66	0.67	0.75	0.58	0.73	0.74	0.64	0.81	0.61	0.71	0.58	0.58	0.53	0.5	0.54	0.69	0.44	0.5	0.3	0.38	0.4	0.34									
R24	0.61	0.62	0.7	0.54	0.68	0.69	0.59	0.77	0.56	0.66	0.53	0.53	0.48	0.46	0.5	0.64	0.4	0.45	0.25	0.34	0.36	0.3	0.18								
R25	0.65	0.66	0.74	0.58	0.72	0.73	0.63	0.81	0.6	0.7	0.57	0.57	0.52	0.5	0.53	0.68	0.44	0.49	0.35	0.34	0.36	0.27	0.4	0.36							
R26	0.66	0.66	0.75	0.58	0.72	0.74	0.63	0.81	0.6	0.7	0.57	0.58	0.53	0.5	0.54	0.68	0.44	0.49	0.4	0.43	0.45	0.39	0.45	0.41	0.45						
R27	0.71	0.72	0.8	0.63	0.77	0.79	0.68	0.74	0.65	0.76	0.63	0.63	0.58	0.55	0.59	0.74	0.56	0.65	0.58	0.61	0.63	0.57	0.63	0.59	0.63	0.63					
R28	0.84	0.84	0.93	0.76	0.9	0.92	0.81	0.82	0.78	0.88	0.75	0.75	0.71	0.68	0.72	0.86	0.69	0.78	0.71	0.74	0.76	0.7	0.76	0.72	0.76	0.76	0.69				
R29	0.73	0.74	0.82	0.65	0.8	0.81	0.71	0.76	0.68	0.78	0.65	0.65	0.6	0.57	0.61	0.76	0.58	0.67	0.6	0.63	0.65	0.59	0.66	0.61	0.65	0.65	0.36	0.71			
R30	0.61	0.62	0.7	0.53	0.67	0.69	0.58	0.76	0.55	0.66	0.53	0.53	0.48	0.45	0.49	0.64	0.18	0.48	0.41	0.44	0.46	0.4	0.47	0.42	0.46	0.46	0.58	0.71	0.6		
R31	0.65	0.66	0.74	0.57	0.72	0.73	0.62	0.8	0.6	0.7	0.57	0.57	0.52	0.49	0.53	0.68	0.43	0.49	0.39	0.42	0.44	0.38	0.45	0.4	0.44	0.33	0.62	0.75	0.65	0.46	
R32	0.63	0.63	0.72	0.55	0.69	0.71	0.6	0.78	0.57	0.67	0.54	0.54	0.5	0.47	0.51	0.65	0.41	0.46	0.37	0.4	0.42	0.36	0.42	0.38	0.42	0.27	0.6	0.73	0.62	0.43	0.3

Fig-3 Pair-wise dissimilarity matrix based on simple matching coefficient of 32 rice genotypes derived from SSR markers data

Among the polymorphic markers, 3 produced two alleles each, 2 produced three alleles each, 5 generated four alleles each and only one produced six alleles [Table-3]. The number of alleles per locus ranged from 2 (RM11, RM25 and RM552) to 6 alleles (RM447) with an average of 3.45 alleles across 11 *loci* obtained in the study. A total of 342 alleles were generated and 135 of these alleles were polymorphic with 38.34% polymorphism and the rest 207 alleles were monomorphic with 60.52% monomorphism. The highest percentage of polymorphism was recorded in RM447 (69.44%), and the lowest polymorphism was in RM484 (6.67%). The Polymorphic Information Content (PIC) was employed for each locus to assess the information of each marker and its discriminatory ability and it is a reflection of allele diversity and frequency among varieties. PIC values ranged from 0.123 to 0.750 with an average of 0.428. Based on the PIC value, 2 primers, RM447 (0.750) and RM277 (0.618) were found to be most informative. The highest PIC value 0.750 was obtained for RM447 followed by respectively RM277 (0.618), and RM316 (0.585). PIC value revealed that RM447 was considered as best marker or most informative for 32 genotypes under study. Heterozygosity (He) value ranged from 0.127 (RM484) to 0.782 (RM447). Pairwise estimates of similarity matrix ranged from 0.18 to 0.98. Similar findings were reported by [7] and [8]. [9] used 24 SSR markers and reported that the PIC values varied widely among *loci* and ranged from 0.239 to 0.765 with an average of 0.508, hence the findings of the present investigation were in accordance. The cluster analysis by using UPGMA algorithm indicated that all the 32 rice genotypes were grouped into three Clusters (I, II and III) [Table-4].

Table-4 *Distribution of 32 rice genotypes into different clusters*

S	Genotype code.	Genotypes	Cluster	Sub-cluster	Sub-sub-cluster
1	R16	Tai Sanghan	I	I A	I A I
2	R11	IC-394788			
3	R6	IC-326284			
4	R7	IC-342353			
5	R5	IC-323781			
6	R15	IC-465275			
7	R13	IC-464661			
8	R12	IC-464363			I A II
9	R9	IC-342369			
10	R14	IC-464684			
11	R10	IC-351517		I B	I B I
12	R1	IC-89071			
13	R4	IC-323776			
14	R3	IC-137401			I B II
15	R2	IC-137342			
16	R24	CT3-D-11	II	II A	II A I
17	R23	CT3-D-10			
18	R19	CT3-D-4			
19	R25	CT3-D-12			
20	R22	CT3-D-9			
21	R21	CT3-D-7			
22	R20	CT3-D-6			
23	R32	Chakhao Amubi			
24	R26	Mantup Chakhao			
25	R31	Chakhao Poireiton			
26	R18	CT3-D-2			II A II
27	R30	Ja-Pnah		II B	
28	R17	CT3-D-1			
29	R28	Faisenbuman Chakhao	III	III A	
30	R8	IC-342368			
31	R29	Bahadur		III B	
32	R27	Kaunglauny Chakhao			

The tree diagram for 32 genotypes expressing the genetic distances based on the 11 SSR markers data was clustered using the software called Darwin 6.0.15 (Figure 2). Cluster I with 15 genotypes (IC-89071, IC-137342, IC-137401, IC-323776, IC-323781, IC-326284, IC-342353, IC-342369, IC-351517, IC-394788, IC-464363, IC-464661, IC-464684, IC-465275 and Tai Sanghan) is the largest cluster. Cluster II is the second largest cluster with 13 genotypes (CT3-D-1, CT3-D-2, CT3-D-4, CT3-D-6, CT3-D-7, CT3-D-9, CT3-D-10, CT3-D-11, CT3-D-12, Mantup Chakhao, Ja-Pnah, Chakhao Poireiton and Chakhao Amubi) and Cluster III consisted of 4 genotypes (IC-342368, Kaunglauny Chakhao, Faisenbuman Chakhao and Bahadur).Cluster I was subdivided into two sub-clusters *viz*., I A and I B. Sub-cluster I A was again grouped into sub-sub-clusters I A I (7 genotypes) and I A II (3 genotypes) and similarly I B into I B I (3 genotypes) and I B II (2 genotypes). The genotypes falling under I A I was Tai Sanghan, IC-394788, IC-326284, IC-342353, IC-323781, IC-465275 and IC-464661. I A II consisted of IC-464363, IC-342369 and IC-464684. Similarly, I B I consisted of IC-351517, IC-89071 and IC-323776. On the other hand, I B II comprised of IC-137401 and IC-137342. On the same outline, Cluster II was again subdivided into sub-clusters II A and II B where II A was further grouped into sub-sub-clusters II A I and II A II. The genotypes falling under II A I was CT3-D-11, CT3-D-10, CT3-D-4, CT3-D-12, CT3-D-9, CT3-D-7, CT3-D-6, Chakhao Amubi, Mantup Chakhao and Chakhao Poireiton. Similarly, II A II consisted of a single genotype CT3-D-2. On the other hand, II B comprised of two genotypes namely Ja-Pnah and CT3-D-1.

Lastly, Cluster III was subdivided into sub-clusters III A and III B having 2 genotypes each. III A consisted of Faisenbuman Chakhao and IC-342368 whereas III B consisted of Bahadur and Kaunglauny Chakhao. The genetic similarity between the 32 rice genotypes was assessed and it ranged from 0.18 to 0.98 obtained through pairwise dissimilarity matrix based on simple matching coefficient of 32 rice genotypes derived from SSR markers data (Figure 3). The highest genetic similarity of 0.18 was observed between the genotypes CT3-D-6 and CT3-D-7; CT3-D-10 and CT3-D-11; and also between CT3-D-1 and Ja-Pnah. The least genetic similarity (0.98) was observed between IC-137401 and IC-342368, followed by IC-342368 and IC-326284 (0.97) where they could be used as two contrasting parents in any desired breeding programme considering the trait under selection. The above finding was in conformity with the finding of [10].

Application of research: The present investigation is only a minute step which involved only 32 genotypes but it gives a clear picture of many desirable traits that can be explored and tapped as well as to safeguard its integrity and its origin. Potential aromatic rice genotypes identified can be used as parents in any breeding programme depending upon the choice of breeder.

Research Category: Genetics and Plant Breeding

Acknowledgement / Funding: Authors are thankful to College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, (Imphal), Umiam, 793103, Meghalaya, India

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Research project name or number: M.Sc. (Agri.) Thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Sample Collection: The 32 rice genotypes were collected from Manipur, Meghalaya and Mizoram as well as received from ICAR-NBPGR Regional Station, Shillong and grown in a completely randomized design with three replications at the Research cum Instructional Farm, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Imphal, Umiam, 793103, Meghalaya, India during *kharif* season of 2017.

Cultivar / Variety / Breed name: Rice (*Oryza sativa* L.)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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