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MICROSATELLITE MARKER-BASED MOLECULAR CHARACTERIZATION OF SMALL AND MEDIUM-GRAINED AROMATIC RICE GERMPLASM OF ODISHA, INDIA

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SUMMARY

Aromatic rice is a kind of specialty rice of the world and is nature's gift to Indo-Pak region. The study aims to evaluate the genetic diversity and relationship among 40 aromatic rice comprising 34 small and medium-grained aromatic rice along with 6 long-grained Basmati rice through microsatellite marker (SSR) analysis using 24 primer-pairs, of which 22 (91.6%) were polymorphic. In total, 51 alleles were detected for 22 polymorphic primer-pairs, with an average of 2.3 alleles per locus. Polymorphism Information Content (PIC) values ranged from 0.05 to 0.57 with an average of 0.33. Four SSR loci revealed PIC values higher than 0.50 and are RM224, RM257, RM241 and RM217. The genotype 'Ganjeikalli' discriminated from rest of genotypes and the basmati-types distinguished from non-basmati types by SSR markers, 'RM249' and 'RM104', respectively. Dendrogram based on the cluster analysis by microsatellite polymorphism, grouped 40 aromatic rice genotypes into 2 major clusters at simple matching (SM) coefficient value of 0.48. Both cluster I and II were divided into 2 subclusters at SM coefficient value of 0.66 and 0.76 respectively, and are further formed sub-subclusters at below subcluster level on basis of eco-geographic regions of Odisha. Clustering pattern obtained in the study revealed that individuals present in cluster-I was more diverse than cluster-II. In subcluster II (i), genotype-pairs Jhingisiali and DP-24, 3114-1 and IC-257505, IC-283204 and IC-283311 were showed extreme similarity (100%) between them. Principal Coordinate Analysis (PCoA) explained that the amount of genetic variation present in SSR molecular data was 63.4% through its cumulative value of first 3 coordinates. Based on this study, the use of SSR marker as a tool to evaluate genetic diversity and their relatedness, which is essential for varietal identification, classification, purity maintenance and conservation of indigenous scented rice germplasm, was elucidated.

Key words: Aromatic rice, molecular characterization, SSR marker, cluster analysis, principal coordinate analysis

Key findings: Four SSR loci (RM224, RM257, RM241 and RM217) revealed higher PIC (> 0.50) and H_e (> 0.60) values could be useful for genetic diversity studies of aromatic rice. Several SSR markers identified for its discriminating power (RM249, RM216, RM228 and RM223), more efficient in unraveling aromatic rice genotypes (RM164, RM242, RM247 and RM19) and for its distinguishing power between basmati-types and non-basmati aromatic rice (RM104) could be useful for future breeding programs.

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INTRODUCTION

Rice (*Oryza sativa* L.) belongs to Poaceae family. It is a staple food for more than half of the world population comprising from Asia and Africa. The small genome size (430 Mb) of rice made it an ideal plant for grass genetics and genomics (Causse *et al.*, 1994). Basmati rice is a kind of specialty rice of the world and is nature's gift to Indo-Pak region. At global level, Basmati rice has high demand and premium price owing to its characteristic features such as pleasant strong aroma, excellent slender grain length with remarkable elongation of kernel length after cooking (Bhasin, 2000). Hundreds of indigenous small and medium-grained aromatic rice are grown by small group of farmers in different localized pockets of most states of India (Richharia *et al.*, 1965) to meet their own consumption and for socio-religious purposes (Bhagwat *et al.,* 2008; Agnihotri and Palni, 2007). The famous short-grained aromatic rice varieties such as Kalanamak, Dubraj, Gobindbhog, Kalajoha etc., are reported from certain rice growing areas of India.

Generally, among the markers the morphological and biochemical markers were used to study genetic diversity available on rice (Gangashetty *et al.*, 2013; Singh *et al.*, 2010; Glaszmann, 1987). PCR-based molecular markers became a powerful tool for genetic diversity studies in recent years and it assay interpret the genetic variation directly at the level of nucleotide sequence of DNA which is made them insensitive to environmental factors. Among molecular markers, the microsatellites also called as sequence-tagged microsatellite sites (STMS) has a pair of primers which specifically amplifies simple sequence repeats (SSRs) and it also exhibits high level of genetic variation in germplasm lines owing to great amount of differences in repeat number at homologous sites (Yang *et al.*, 1994). As a co-dominant genetic marker, it detects high levels of allelic diversity present in the population (McCouch *et al.*, 1997) and its widespread use in rice for construction of genome map (Temnykh *et al.,* 2000), mapping and tagging of genes (Mishra *et al.,* 2003) and for varietal characterization (Garland *et al.*, 1999) were also reported.

Among aromatic rice, most of the genetic diversity analyses using SSR markers focused mainly on basmati-types (Nagaraju *et al.*, 2002; Singh *et al.*, 2011). Although, a vast amount of diversity available on world aromatic rice collections (Byerlee, 1996), little attempt has been carried out on systematic analysis of genetic diversity on non-basmati types than basmati types. Myint *et al.* (2012) distinguished the Myanmar aromatic rice varieties from non-Myanmar varieties (collected from south and south-east) by obtaining 2 subclusters, '5B' and '5A', respectively, in isozyme based varietal group 'V' by using microsatellites. The deployment of SSR markers for exploitation of useful traits present among 41 rice genotypes representing different parts of India was elucidated by Pachauri *et al.* (2013). The correlation and path coefficient analysis of non-basmati aromatic rice genotypes revealed the existence of association among yield and yield attributing traits such as number of tillers per plant, number of productive tillers per plant, panicle weight, test weight at both genotypic and phenotypic level (Gangashetty *et al.*, 2013). Sivaranjani *et al.* (2010) distinguished 30 basmati-types from 16 non-basmati type aromatic rices by using either single or combination of 2 microsatellite markers.

Eco-geographic locations of Odisha harbor several indigenous aromatic rice, which are less exploited and investigated. Singh *et al.* (2010) classified 20 genotypes belonging to different parts of Orissa on basis of biometrical parameters using numerical taxonomic analysis. Forty-eight aromatic rice genotypes collected from different areas of Odisha studied for genetic diversity and their relationships using SSR (Meti *et al.*, 2013) and ISSR markers (Samal *et al.*, 2014) revealed presence of good amount of intra and inter genetically variation useful to breeders to improve the aromatic rice varieties through selective/ cross breeding programs and also useful to protect the germplasm under Intellectual Property Rights. Hence, the present study was formulated to evaluate the genetic diversity using SSR markers and also to develop DNA profiles of indigenous small and medium-grained aromatic rice germplasm representing diverse eco-geographic locations of Odisha.

MATERIALS AND METHODS

Experimental material

The experimental material consisted of 40 aromatic rice genotypes comprising 34 small and medium-grained aromatic rices along with 6 long-grained basmati rice, assembled mainly from Odisha state of India, representing major agro-ecological conditions of the region. A passport data of these accessions are given in Table 1 and source area of genotypes shown in Figure 1.

DNA extraction and Selection of primer

The total genomic DNA of aromatic rice genotype was isolated from bulked leaf samples (~ 15 mg each) from 5 plants (1 month-old) using CTAB method described by Saghai-Maroof *et al.* (1984) with minor modification. The DNA was spooled out, washed twice with 70% ethanol, and dissolved in TE (10 mM Tris,0.1 mM EDTA, pH 8.0) containing 25 µg/ml RNase-A, incubated at 37º C for 30 min and extracted with chloroform:isoamyl alcohol (24:1 v/v). DNA was checked for its quality and quantity by 1% agarose gel electrophoresis using a standard containing 100 ng/ µl genomic lambda DNA. Thirty primers were screened to identify suitable primers for molecular diversity analysis of 40 aromatic rice genotypes. The primers were selected from the sequence information obtained from the website of Cornell University (Hong-Liang *et al.*, 2004). Among 30 primers, 24 primer-pairs were selected for present study and the details of those primers are listed in Table 2.

PCR amplification and Gel electrophoresis

The standard procedure was used for PCR amplification, agarose gel (2.4%) preparation, gel electrophoresis and its documentation.

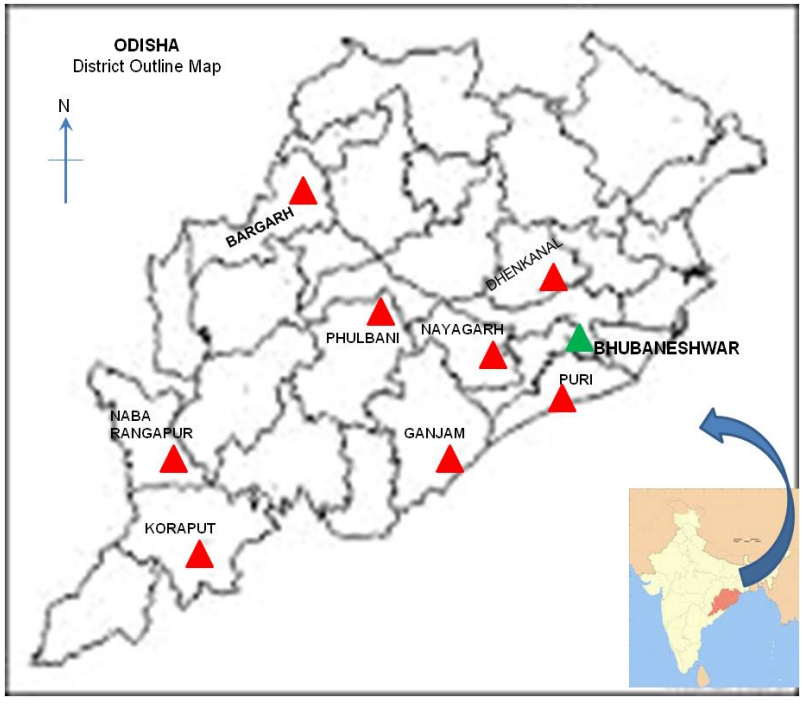

Figure 1. Map showing locations of small and medium grained aromatic rice genotypes belongs to Odisha state of India (Red triangle indicates the source area of genotypes) and the boundary of Odisha state in outline map of India was depicted in inner side.

Table 1. Passport information of 40 aromatic rice genotypes with line number, genotype name, accession number and its place of origin.

Line No.	Genotypes	Accession	Origin
1.	Dahiya	-	Uttar Pradesh
2.	Seond Basmati (TIB)*	-	Himachal Pradesh
3.	Sonasal	-	Assam
4.	Basmati 370 (TIB)	-	Punjab
5.	Chimbalate basmati (TIB)	-	Himachal Pradesh
6.	Bindli	-	Uttar Pradesh
7.	Sharbati	-	Uttar Pradesh
8.	Pusa sugandh-4 (CIB)**	-	IARI, New delhi
9.	Taraori basmati (TIB)	-	Haryana
10.	Gatia	-	Nabarangapur, Odisha
11.	Jhingisiali	-	Puri, Odisha
12.	Deulabhoga	IC 203544	Puri, Odisha
13.	Maguraphula	IC 373145	Bargarh, Odisha
14.	Nanhu	IC 259033	Odisha
15.	Dhobaluchi	-	Puri, Odisha
16.	Barikuncha	-	Puri, Odisha
17.	Ganjeikalli	-	Puri, Odisha
18.	OR-11	-	Odisha
19.	Gangabali	IC 280574	Koraput, Odisha
20.	Tulaisiphula	IC 256654	Puri, Odisha
21.	Dubraj	IC 256622	Nabarangapur, Odisha
22.	Krishnabhog	IC 283244	Puri, Odisha
23.	Acharmati	-	Nabarangapur, Odisha
24.	OR-18	-	Odisha
25.	3114-1	-	Odisha
26.	Krishnabhog	IC 257018	Puri, Odisha
27.	Basumati	IC 256871	Puri, Odisha
28.	Kendragali	-	Odisha
29.	Kalagira	IC 257505	Dhenkanal, Odisha
30.	Kalagira	IC 283204	Dhenkanal, Odisha
31.	Nadiarasa	IC 283311	Ganjam, Odisha
32.	Basumati	IC 256907	Puri, Odisha
33.	Saragadhuli	-	Puri, Odisha
34.	DP-21	-	Odisha
35.	DP-22	-	Odisha
36.	Tulasibasa	IC 280552	Nayagarh, Odisha
37.	DP-24	-	Odisha
38.	Karpurakanti	IC 256684	Phulbani, Odisha
39.	Basuabhog	IC 256682	Phulbani, Odisha
40.	Kalakrishna	IC 203545	Dhenkanal, Odisha

* TIB – Traditional Indian Basmati; **CIB – Cross-bred Indian Basmati

Table 2. Details of the 24 SSR markers used for molecular characterization of 40 aromatic rice genotypes [linkage group, SSR motif, annealing temperature (AT), number of alleles detected, allele size, polymorphism information content (PIC) and expected hetero-zygosity (H_e)]

Marker	Linkage group	SSR motif	AT (^{0}C)	Alleles detected*	Allele size** (bp)	PIC value	Expected heterozygosity (H_e)
RM104	1	(GA)9	50	2	200-250	0.16	0.18
RM263	2	(GA)34	50	2	180-200	0.34	0.44
RM53	2	(GA)14	50	2	150-175	0.20	0.22
RM25	3	(GA)18	50	2	100-125	0.36	0.47
RM218	3	(GA)24	52	2	120-150	0.37	0.49
RM 261	4	(GA)8	52	1	120-125	0.00	0.00
RM241	4	(GA)31	50	3	100-170	0.52	0.59
RM233B	5	-	48	2	150-170	0.25	0.29
RM164	5	(GA)16	40	2	250-300	0.35	0.46
RM249	5	(GA)14	50	2	100-125	0.05	0.05
RM217	6	(GA)20	46	3	120-160	0.50	0.58
RM3	6	(GA)25	50	2	100-125	0.31	0.38
RM11	7	(GA)17	55	2	120-150	0.33	0.42
RM234	7	(GA)17	52	2	120-150	0.35	0.46
RM223	8	(GA)25	54	3	150-200	0.21	0.22
RM3262	8	(CT)13	50	1	170-175	0.00	0.00
RM257	9	(GA)24	52	3	100-175	0.55	0.63
RM242	9	(GA)26	52	2	200-225	0.37	0.49
RM216	10	(GA)18	48	2	150-175	0.16	0.18
RM228	10	(GA)36	48	3	100-150	0.14	0.14
RM222	10	(GA)18	50	2	200-220	0.31	0.38
RM224	11	(GA)13	44	3	150-180	0.57	0.64
RM19	12	(ATC)10	46	2	200-225	0.34	0.44
RM247	12	(GA)16	48	3	150-200	0.48	0.57
Total				51***		0.33***	0.39***

*Average number of alleles per primer (polymorphic primer) = 2.3
**Range of allele size = 100 to 300 bp
***Excluding mono-morphic primers

Data analysis

For marker data analysis, the amplification products were scored as present (1) or absent (0). The polymorphism information content (PIC) and hetero-zygosity (H_e) value was calculated using online software available at website: http://www.liv.ac.uk/~kempsj/pic.html. The Un-weighted Paired Group Method with Arithmetic means (UPGMA) based Simple Matching Coefficient was used for cluster analysis. This computation was performed by using NTSYS-pc 2.2 (Rohlf, 2005). The Principal Coordinate Analysis (PCoA) was also carried out by using molecular data. Two-dimensional scatter plot was generated to represent the accessions with their genetic variability.

RESULTS

SSR data analysis

Among the 24 SSR primer-pairs used in the present study, 22 (91.6%) were polymorphic, while 2 primers revealed mono-morphic patterns. In total, 51 alleles were detected for the 22 polymorphic primers, with an average of 2.3 alleles per locus.

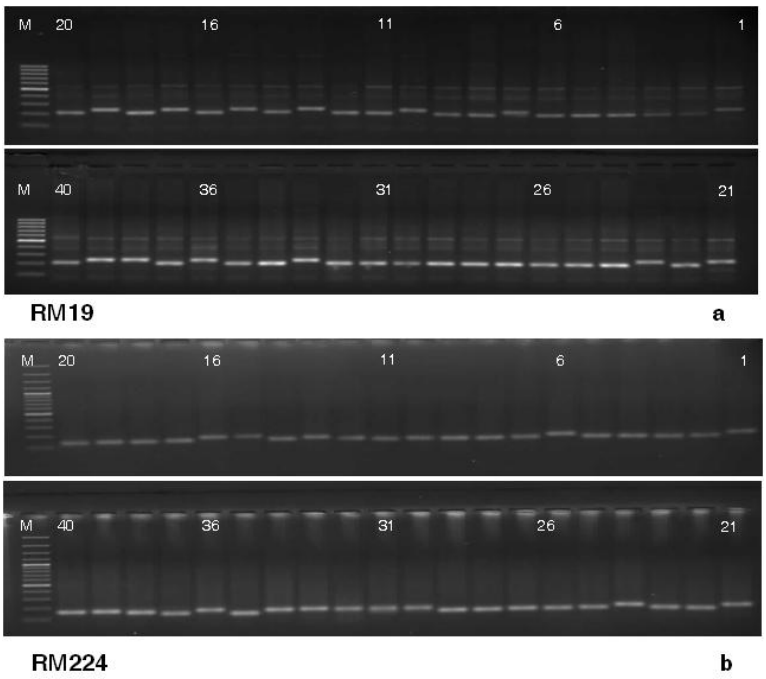

Figure 2. SSR profiles of 40 aromatic rice genotypes generated by polymorphic primers, RM19 and RM224 using bulked DNA in which 'M' denotes marker lane and '1-40' denotes genotypes lane.

The profiles of SSR markers with polymorphism such as RM19 and RM224 were shown in Figure 2. The number of alleles detected per primer ranged from 1 to 3, whereas, the size of alleles ranged from 100 to 300 bp (Table 2). The PIC values ranged from 0.05 to 0.57 with an average of 0.33. The H_e values ranged from 0.05 to 0.64, with an average of 0.39. Four SSR loci revealed PIC and H_e values higher than 0.50 and 0.60, respectively, and are RM224, RM257, RM241 and RM217 (Table 2).

Cluster analysis

Cluster analysis based on Simple matching (SM) coefficient using UPGMA method clearly distinguished 40 aromatic rice genotypes into 2 major clusters, Cluster I and II at SM coefficient value of 0.48 (Figure 3). Both Cluster I and II are divided into 2 subclusters at SM coefficient value of 0.66 and 0.76, respectively. On basis of eco-geographic region of Odisha, these subclusters were further divided into sub-subclusters at below subcluster level. Bootstrap analysis validated the clustering pattern resulted from marker data. Among 14 genotypes in Cluster I, 13 genotypes (Dahiya, IC-373145, Gatia, Dhobaluchi, IC-256622, Ganjekalli, Acharmati, IC-259033, IC-280574, IC-256682, Saragadhuli, IC-280552 and IC-256684) from Nabarangapur, Phulbani and its adjoining areas formed the subcluster I (i), leaving Sharbati to form a separate subcluster I (ii). Twenty-five genotypes of aromatic rice (Seond basmati, IC-256654, Jhingisiali, DP-24, DP-22, 3114-1, IC-257505, IC-256871, IC-257018, Kendragali, Barikuncha, IC-283244, OR-18, DP-21, IC-283204, IC-283311, IC-203544, IC-256907, IC-203545, Sonasal, Basmati-370, Chimbalate basmati, Pusa sugandh-4, Taraori basmati and Bindli) from Puri and its neighboring areas along with traditional / improved basmati varieties formed Subcluster II (i), while genotype OR-11 itself forms a separate subcluster II (ii). Genotype-pairs Jhingisiali and DP-24, 3114-1 and IC-257505, IC-283204 and IC-283311 were showed 100% similarity with SM coefficient value of 1.00 between them. The small and medium-grained aromatic rice genotypes, Bindli and Sonasal were grouped along with basmati varieties such as Basmati-370, Chimbalate basmati, Pusa sugandh-4 and Taraori basmati.

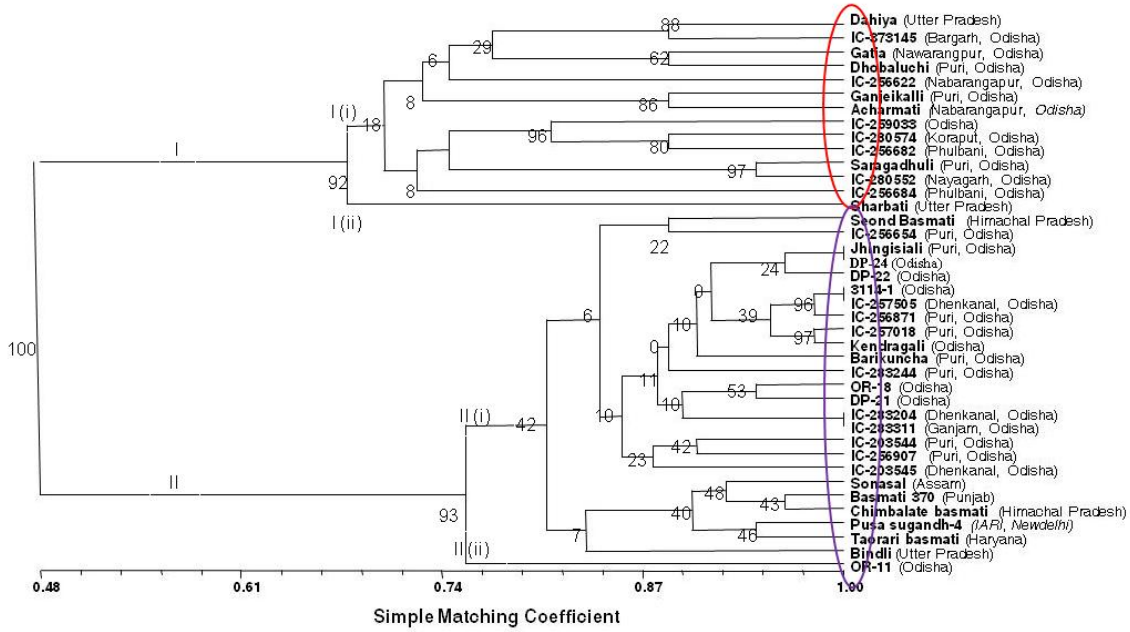

Figure 3. Dendrogram based on UPGMA method using simple matching coefficient measuring genetic similarity among aromatic rice genotypes according to molecular data generated by 24 SSR markers with Bootstrap values.

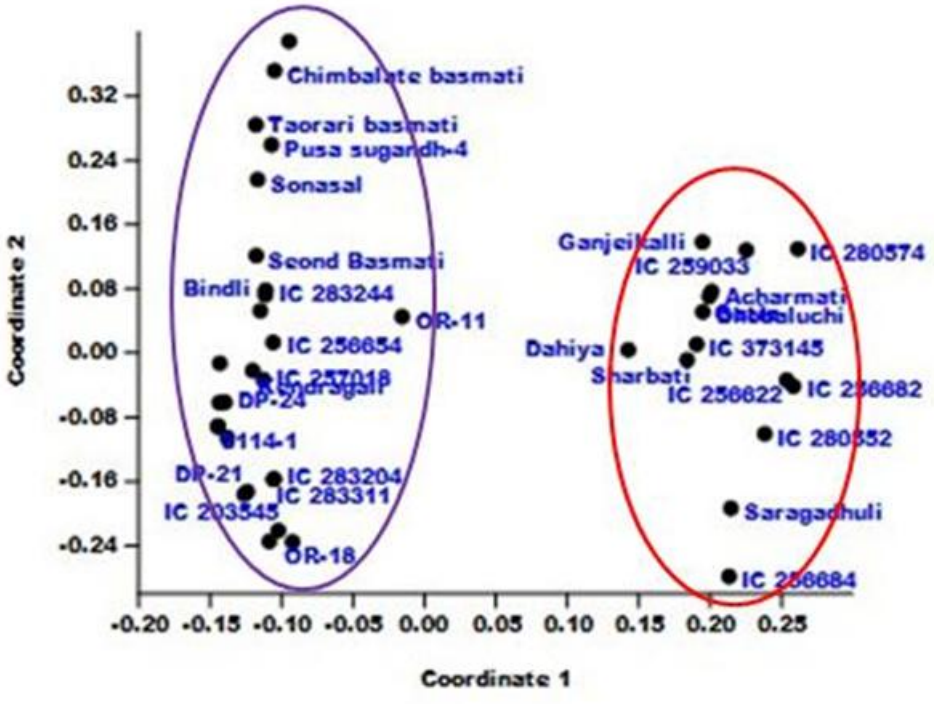

Figure 4. Two-dimensional principal coordinates analysis (PCoA) ordination of 40 aromatic rice genotypes according to molecular data generated by 24 SSR markers which validates the pattern obtained in the cluster analysis.

Principal Coordinate Analysis (PCoA)

PCoA analysis was also performed to determine the genetic relationships among 40 aromatic rice genotypes. Two-dimensional scatter-plot of PCoA ordination for aromatic rice genotypes is presented in Figure 4. The value of variance accounted by PCoA analysis from first 3 most informative principal coordinates was 63.4% which provides the amount of genetic variation present in SSR molecular data derived from aromatic rice genotypes in the study. First, second and third principal coordinates explained about genetic variation present in aromatic rice genotypes as 47.7%, 9.5% and 6.2% respectively with cumulative variation of 63.4%.

DISCUSSION

The center of diversity of aromatic rice is considered as foothills of Indian Himalayas comprising the states of Uttar Pradesh and Bihar and Terai regions of the Nepal (Khush, 2000). However, indigenous small and medium-grained aromatic rice is grown in several states of India with particular geographic delineation for their cultivation than long-grained basmati types (Singh *et al.*, 1997). Although, a huge number of collections are available on aromatic rice, little attempt on systematic analysis of genetic diversity has yet been carried out. Molecular markers could be used to analyze genetic diversity available among rice genotypes. But, there is no concrete rule to estimate genetic diversity with genome-wide coverage of species and also on optimum number of markers to be used to identify core variability available in germplasm collections. However, identification and classification of aromatic rice cultivars and germplasm collections could be made precisely by utilizing more number of polymorphic SSR markers available in public domain owing to its characteristic features such as co-dominant nature, reproducibility, reliability and wide genome coverage. Forty aromatic rice genotypes, including basmati varieties, characterized for molecular diversity by using 24 SSR markers in present study revealed a considerable amount of genetic polymorphism among them.

Marker based genetic diversity

In the present study, SSR marker profiling was found capable of revealing the genetic polymorphism available among 40 aromatic rice genotypes. Average number of alleles per locus (2.3) obtained in the study was comparable with result reported by Meti *et al.* (2013) who studied 48 aromatic rice genotypes from different areas of Odisha using 12 SSR primers which detected 2.3 alleles per locus and also obtained a total of 28 alleles which was markedly lower than the current result (51 alleles). Further, Jain *et al.* (2004) obtained 62 (26.4%) of total 235 alleles from only basmati and other scented rice germplasm accessions among the total collections using 30 SSR markers and number of alleles per locus ranged from 3 to 22 with an average of 7.8 which was obviously higher than present study. Number of alleles detected per locus and nature of alleles present in the population are fundamental sign of presence of rich allelic diversity. On the basis of allelic diversity, utility of cultivar or germplasm collection in future breeding programs could be decided. Further, this kind of genetic polymorphism in SSR markers is directly related to use of huge number of diverse cultivars and microsatellite loci under study. The PIC value, a reflection of allelic diversity and frequency among the rice genotypes analyzed were generally high for all the SSR loci tested. Among 4 SSR loci identified with high PIC and H_e values in the present study, RM224 and RM257 are noteworthy due to their relatively higher PIC (0.57 and 0.55) and H_e (0.64 and 0.63) values, respectively. The higher range and average of PIC values of SSR marker reported by Meti *et al.* (2013), Sivaranjani *et al.* (2010) and Jain *et al.* (2004) for aromatic rice genotypes than present study [0 to 0.57 and 0.33] were: [0 to 0.74 and 0.58], [0.17 to 0.73 and 0.53] and [0.20 to 0.90 and 0.60] respectively.

One of the most important applications of molecular diversity studies is to identify a suitable marker which can differentiate a genotype from the remaining genotypes, but this is difficult to achieve in a closely related set of genotypes. Although, current collection of genotypes is closely related, few SSR markers

were identified with more efficiency in the present study to discriminate them from rest of collections. A SSR marker, 'RM249' located on chromosome '5' differentiated the genotype 'Ganjeikalli' from rest of genotypes. Another SSR marker, 'RM104' located on chromosome '1' distinguished long-grained traditional/ improved Basmati-types (Basmati-370, Chimbalate basmati, Pusa sugandh-4 and Taraori basmati) from small and medium-grained non-basmati aromatic rices. The genotypes Sharbati, Ganjeikalli, IC-256622, IC-283244 and Acharmati were discriminated from others by SSR marker 'RM223' located on chromosome '8', whereas, the genotypes [IC-259033, IC-280574] and [IC-256622, Saragadhuli, IC-280552] were differentiated by SSR markers, 'RM216' and 'RM228' located on chromosome '10'. Sivaranjani *et al.* (2010) discriminated 30 basmati-types from 16 short and medium-grained aromatic rices belonging to different parts of India by using either single (RM28102) or the combination of 2 (RM577+RM30) microsatellite markers. Saini *et al.* (2004) evaluated the traditional Basmati, cross-bred Basmati and non-Basmati (indica and japonica) rice varieties using 3 DNA markers such as AFLP, ISSR and SSR. All the 3 marker systems generated higher levels of polymorphism and could distinguish between all the 18 rice cultivars.

Cluster analysis

The UPGMA dendrogram obtained by using Simple matching (SM) coefficient index value on basis of SSR data revealed a genetic relationship among 40 aromatic rice genotypes by clustering together. Unlike the nearest or furthest neighbour (single or complete linkage) method, a clustering pattern based on UPGMA method has advantage over them by utilizing information available regarding all pairs of distances in the cluster. The SM coefficient value ranging from 0.45 to 1.00 indicated an existence of considerable amount of variability among aromatic rice genotypes. Revelation of huge range of values of genetic similarity among cultivars by SSR markers offers greater confidence for assessment of genetic diversity and relationship (Chakravarthi and Naravaneni, 2006). Similar to current clustering pattern was also obtained by Meti *et al.* (2013) for 48 aromatic rice collection from Odisha using 12 SSR markers, which separated the genotypes into 2 major clusters at 49% genetic similarity using jaccard's similarity index. Clustering pattern obtained in the present study revealed that individuals in cluster-I was more diverse than cluster-II which proved through low SM coefficient index value between genotypes in cluster-I. The SSR markers located on chromosome '5' (RM164), '9' (RM242) and '12' (RM247 and RM19) were more efficient in unraveling the 40 aromatic rice genotypes into 2 major clusters, I and II than the SSR markers located on chromosome '2' (RM263), '3' (RM25), '6' (RM217) and '10' (RM222).

At below subcluster level, the majority of genotypes showed more than 87% of genetic similarity among them which itself explains about similarity in geographical origin of these genotypes and it also illustrated the existence of narrow genetic base among aromatic rice genotypes. Above 75% genetic similarity between genotypes in cluster analysis for 48 traditional aromatic rice collected from different parts of Odisha using 12 SSR and 24 ISSR markers were obtained by Meti *et al.* (2013) and Samal *et al.* (2014) respectively, which was comparable to current results obtained in present study which also comprised the genotypes from same state. In subcluster-I (i), the high level of genetic similarity (96%) was showed by genotypes Saragadhuli and IC-280552, which followed by genotypes Dahiya and IC-373145, Gatia and Dhobaluchi, Ganjeikalli and Acharmati, IC-280574 and IC-256682 (90%). At molecular level, the genotype 'Sharbati' has 70% similarity with genotypes in Subcuster-I (i).

In subcluster-II (i), genotype-pairs Jhingisiali and DP-24, 3114-1 and IC-257505, IC-283204 and IC-283311 were found with 100% genetic similarity (SM coefficient index of 1.00) which explains about their similarity at gene level and it could possibly be due to local selections made by the farmers from a single landrace (Choudhury *et al.*, 2001) or cultivars belonging to similar genetic background (Chakravarthi and Naravaneni, 2006). The genotype IC-256871 has 98% genetic similarity with genotype-pair, (3114-1 and IC-257505) and

the genotypes (IC-257018 and Kendragali) has also showed same level of similarity. Genotypes OR-18 and DP-21 showed 95% genetic similarity at molecular level, whereas, 91% similarity has shown by genotypes belonging to Puri, IC-203544 and IC-256907. The level of genetic similarity exhibited by genotypes 'Sonasal' and 'Bindli' were 92 and 85% with genotypes (Basmati 370 and Chimbalate basmati) and (Sonasal, Basmati 370 and Chimbalate basmati), respectively. Genotype OR-11 showed 75% genetic similarity with genotypes present in Subcluster-II (i).

Except Dahiya, all genotypes in subcluster-I (i) were belonging to Odisha state, in which, 5 genotypes (IC-373145, Gatia, IC-256622, Acharmati and IC-280574) from Nabarangapur and its adjacent areas formed clusters at below subcluster level by leaving the rest genotypes (Dhobaluchi, Ganjekalli, IC-259033, IC-256682, Saragadhuli, IC-280552 and IC-256684) from Phulbani and Puri areas to form another cluster. Although, there was a differences in eco-geographic origin, the inclusion of these genotypes into subcluster I (i) might be due to their genetic similarity at molecular level among genotypes present in below subcluster level. The genotypes belonging to Nabarangapur and neighboring areas (Gatia, IC-256622, IC-280574, IC-256682, IC-256684) and Sharbati were differentiated from other genotypes of Odisha by SSR marker 'RM233B' located on chromosome '5'. The *indica* variety, Sharbati (varietal group III, isozyme-based polymorphism), has little or no aroma and grain elongation characteristic of Basmati rice, was quite divergent from traditional basmati, cross-bred basmati, aromatic rice and other rice varieties (Pal *et al.*, 2004), which made it to form a separate Subcluster I-(ii) in Cluster-I with SM coefficient value of 0.66. The SSR marker, 'RM223' located on chromosome '8' differentiated the genotype 'Sharbati' from rest of genotypes with more efficiency than the SSR markers located on chromosome '2' (RM53) and '5' (RM233B).

A degree of genetic similarity between genotypes of aromatic rice revealed from their clustering pattern could be useful to distinguish the basmati types from non-basmati types. At SM coefficient value 0.82, the Basmati rice varieties formed separate Sub-subclusters from aromatic rice genotypes of Odisha which explains about genetic similarity and relationships among them at molecular level. Similarity in geographical origin and vital quality parameters made the small and medium-grained aromatic rice genotypes, Bindli and Sonasal to group with basmati varieties such as Basmati-370, Chimbalate basmati, Pusa sugandh-4 and Taraori basmati within subcluster II (i). On Cluster-II, a small-grained aromatic rice genotype named OR-11 formed individually a separate cluster at subcluster level (at SM coefficient value, 0.76) which could be owing to its comparable nature with traditional and improved basmati varieties in cases of quality characteristics such as strong aroma and kernel elongation after cooking (Venkatesan *et al.*, 2011).

PCoA analysis

PCoA analysis is a kind of multivariate analysis which forms grouping of genotypes on the basis of similarity coefficients or variance-covariance values and it is expected to be more informative about differentiation of major groups. Amount of genetic variation present in aromatic rice genotypes through SSR data was explained by first 3 principal coordinates as 63.4% and first, second and third principal coordinates were 47.7%, 9.5% and 6.2% respectively. Its suitability to clustering of genotypes was revealed through its ability in differentiating genotypes into 2 major clusters in the present study. The groupings identified through PCoA analysis were almost similar to those identified in cluster analysis which explains about conformity of results obtained from the study.

CONCLUSION

The present study revealed a considerable genetic diversity present in the 40 aromatic rice genotypes mainly belongs to Odisha state of India at molecular level and a limited number of SSR markers efficiently grouped these genotypes into 2 clusters on basis of eco-geographic origin. SSR markers such as RM249, RM216, RM228 and RM223 could be useful to

breeders for discriminating different aromatic rice genotypes and RM104 will be useful to distinguish basmati-types from non-basmati aromatic rices. We advocate a survey on basis of extensive exploration study and collection of small and medium-grained aromatic rice genotypes from different agro-climatic and eco-geographical regions of India in order to conserve a maximum diversity available in those areas. Finally, conservation of biodiversity of small and medium-grained aromatic rice genotypes and its utilization in development of commercial basmati varieties through crop improvement or breeding programs are linked processes.

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