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Genetic diversity analysis in aromatic rice genotypes using microsatellite based simple sequence repeats (SSR) marker

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The microsatellite or simple sequence repeat (SSR) markers were used to determine the allelic diversity and relationship among 48 traditional indigenous aromatic rice germplasm grown under Eastern part of India. Out of 30 primers, 12 primers showed DNA amplification and polymorphism among 48 aromatic rice genotypes. A total of 28 bands appeared by using 12 SSR primers in 48 aromatic rice varieties/landraces. The number of alleles per locus ranged from 1 to 5 with an average 2.08. Out of 28 bands, 25 bands were polymorphic and three were monomorphic bands. The results reveal that all the tested primers showed distinct polymorphism among the landraces/varieties indicating the robust nature of SSR markers. Most of the primers showed highest polymorphic information content (PIC). Phenotypic characteristics are significantly correlated with genotypic characters. The cluster analysis indicates that the 48 traditional indigenous aromatic rice genotypes were grouped into two major clusters. Among the two major clusters, one cluster had 11 varieties and the second cluster had 37 varieties on the basis of the group of land races. Based on this study, the larger range of similarity values using SSR markers provides greater confidence for the assessment of genetic relationships among the varieties. The information obtained from the SSR profile helps to identify the variety diagnostic markers in 48 traditional indigenous aromatic rice genotypes. Significant genetic variation at maximum number of loci between varieties indicates rich genetic resources in rice. The intra and inter genetically variation might be useful for breeders to improve the aromatic rice varieties through selective breeding and cross breeding programs and also protect these unique germplasm under Intellectual Property Rights (IPR).

Key words: Aromatic Rice, *Oryza sativa*, DNA profile, diagnostic polymerase chain reaction simple sequence repeat (PCR-SSR) marker, polymorphism, genetic diversity.

INTRODUCTION

Rice (*Oryza sativa* L.) (2n = 24) belonging to the family, Poaceae and subfamily, Oryzoidea is the staple food for

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Abbreviations: CBT, Conservation of biological diversity; CTAB, N-cetyl-N, N, N-trimethyl ammonium bromide; PCA, principal component analysis; SSRs, simple sequence repeats; PCR, polymerase chain reaction; PIC, polymorphic information content.

half of the world's population and occupies almost one-fifth of the total land area covered under cereals. It is one of the very few crop species endowed with rich genetic diversity which account over 100,000 landraces and improved cultivars. Being the secondary centre of origin of cultivated rice, Odisha, India has the distinction of possessing about 10,000 to 15,000 traditional rice varieties out of 45,000 to 50,000 found in the world (Ray, 2007). Among these traditional rice varieties, land races of aromatic rice bear special significance because of their special flavour and economic value in the present globalized era (Chaudhury et al., 2001). The improvement of indigenous small and medium grained aromatic rice, which possesses outstanding quality like aroma, kernel elongation after cooking, fluffiness and taste were somewhat neglected as they lacked export value. Little attention has been paid to their improvement except for sporadic reports on germplasm evaluation and genetics of some quality trait. Scanty information is available on genetic diversity of traditional indigenous aromatic rice germplasm. Therefore, these varieties have to be collected and evaluated for their exploitable genetic variability and conserved. Further, management of the indigenous aromatic rice genetic resources by way of characterisation and documentation helps in protection of these unique bio-resources in accordance with the provision laid out in the 1992 to meet on conservation of biological diversity (CBT). Some of these genotypes are being gradually eroded from their respective places of origin and are on the verge of becoming extinct due to competition from high yielding varieties, difficulties of cultural practices and improper means of storage (Ram et al., 2007).

Among Asian rice growing countries, India is a major producer of many rice varieties such as aromatic rices and old landraces. In the context of global biodiversity loss, India missed several rice varieties/landraces. Therefore, it is highly necessary not only to conserve the landrace genotypes but also to investigate the gene-pool of aromatic rice for breeding purposes of high yielding varieties/landraces in the country. Characterization of varieties based on morphological characters is not very reliable because major characters have low heritability and are genetically complex warranting more precise techniques. For this purpose, identification of different genotypes at molecular level is imperative. The DNA-based markers are promising and effective tools for measuring genetic diversity in plants germplasm and elucidating their evolutionary relationships. They are more reliable, and remain unaffected across different growth stages, seasons, locations 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 co-dominant, easily and distributed throughout the genome. More than 20,000 microsatellite markers have

been mapped to specific locations in rice genome (www.irgsp.org) (Pervaiz et al., 2009). 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 (Nagaraju et al., 2002). Molecular markers have been extensively used to identify genetic variation among rice germplasm (Nagaraju et al., 2002; Yu et al., 2003; Ren et al., 2003; Jain et al., 2004; Zeng et al., 2004; Garris et al., 2005; Shishido et al., 2006; Gao et al., 2005; Jayamani et al., 2007; Sajib et al., 2012). In the present study, a set of 12 microsatellite markers distributed on 12 different chromosomes of rice genome were used for DNA profiling of 48 indigenous aromatic rice genotypes to characterize and detect genetic diversity within these cultivars at molecular level.

MATERIALS AND METHODS

The aromatic rice varieties were collected from the Eastern parts of the India and were maintained in the Rice Research station, Orissa, University of Agriculture and Technology (OUAT), Bhubaneswar, India. 48 elite aromatic rice varieties were selected for phenotypic and molecular analysis (Table 1). The genotypes were grown in Randomized Complete Block Design with three replications during kharif season for three consecutive years. The plot size for each variety was 3.0 x 3 m and a spacing of 20 cm between lines and 10 cm between plants were provided. The standard recommended agronomic practices were followed for raising the crop. Observation on different quantitative characters viz. plant height, days to flowering, panicle length, panicle number, number of fertile grains, 1000 grain weight, yield and other phenotypic characters were recorded. The observations of different quantitative characters were recorded as per DUS (distinctness, uniformity, stability) guidelines.

Isolation of rice genomic DNA

Leaf samples were collected and subsequently stored at -20°C for isolation of genomic DNA. The genomic DNA was extracted from young leaves using N-Cetyl-N, N, N-trimethyl ammonium bromide (CTAB) method described by Doyle and Doyle (1990) with slight modifications. 2 g of fresh leaf material were washed in distilled water and subsequently rinsed with 80% (v/v) ethanol and then ground in liquid nitrogen. 10 ml of preheated extraction buffer [4 % (w/v) CTAB, 0.2% β -mercaptoethanol (v/v), 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl] were then added per 2 g of leaf powder material and incubated for 2 h at 65°C . The lysate was purified with chloroform: isoamylalcohol (24:1). The DNA pellet was resuspended in 200 to 300 μl of Tris-EDTA buffer (10 mM Tris – HCl, 1 mM EDTA, pH = 8.0). DNA was reprecipitated by adding 80% ethanol in the presence of 0.3 M sodium acetate, and pelleted by centrifugation. The pellets were lyophilized and resuspended in TE buffer. The RNA was removed by RNase treatment at 37°C for 1 h. For further purification, DNA solution was extracted once with equal volume of phenol and chloroform: isoamyl alcohol (24:1:1) followed by two extractions with chloroform: isoamylalcohol (24:1). The upper aqueous phase was separated after centrifugation and

Table 1. Aromatic rice varieties and their distribution.

S/N	Genotype	Area of distribution
1	Baranamgomati	Cuttack, Odisha
2	Basnasapuri	Puri, Odisha
3	Basnaparijat	Puri, Odisha
4	Basumati-1	Puri, Odisha
5	Basmatidhan	Keonjhar, Odisha
6	Basumati Bhog	Koraput, Odisha
7	Chatianaki	Cuttack, Odisha
8	Dhobaluchi	Phulbani, Odisha
9	Krishnabhoga	Bolangir, Odisha
10	Manasi-2	Puri, Odisha
11	Nuakalajeera	Puri, Odisha
12	Nuadhusura	Cuttack, Odisha
13	Pimpudibasa	Keonjhar, Odisha
14	Ratnasundari	Kalahandi, Odisha
15	Sujata	Puri, Odisha
16	Srimula	Cuttack, Odisha
17	Thakurbhog	Puri, Odisha
18	Tulasiphada	Puri, Odisha
19	Sheetabhog	Cuttack, Odisha
20	Gopalabhoga	Puri, Odisha
21	Tulasiphulla	Puri, Odisha
22	Bhasumati (P)	Phulbani, Odisha
23	Saragadhulli	Puri, Odisha
24	Mugajai	Bolangir, Odisha
25	Dholabankoi	Cuttack, Odisha
26	Dubraj	Keonjhar, Odisha
27	Dulhabhoga	Keonjhar, Odisha
28	Dangarabasamati	Phulbani, Odisha
29	Gangaballi	Phulbani, Odisha
30	Ganjam local-1	Ganjam, Odisha
31	Ganjam local-2	Ganjam, Odisha
32	Ganjeikalli	Cuttack, Odisha
33	Gatia	Puri, Odisha
34	Heerakani	Dhenkanal, Odisha
35	Jalaka-1	Keonjhar, Odisha
36	Jalaka-2	Keonjhar, Odisha
37	Jaiphulla	Keonjhar, Odisha
38	Kalajeera	Puri, Odisha
39	Kalajauvan	Puri, Odisha
40	Khosakani	Koraput, Odisha
41	Kaminibhoga-1	Bolangir, Odisha
42	Kaminibhoga-2	Bolangir, Odisha
43	Kalikati-2	Kalahandi, Odisha
44	Kalikati-1	Kalahandi, Odisha
45	Kanakachampa	Keonjhar, Odisha
46	Kusumabhoga	Denkanal, Odisha
47	Karpurakranti	Bolangir, Odisha
48	Manasi-1	Puri, Odisha

mixed with 1/10th volume of 3 M sodium acetate. DNA was precipitated by adding two volumes of chilled absolute alcohol, pelleted, dried in vacuum and dissolved in TE buffer. Quantification of DNA was accomplished by analyzing the purified DNA on 0.8% (w/v) agarose gel electrophoresis along side diluted uncut lambda DNA as standard. DNA was further diluted with TE to a concentration

of 20 ng/μl for use in PCR analysis.

PCR amplification and electrophoresis

A set of 12 mapped microsatellite markers distributed on all the 12 chromosomes (McCouch et al., 1997; Temnykh et al., 2000) were used for the analysis of 48 aromatic rice genotypes. The number of markers per chromosome was one. These microsatellite primers were purchased from commercially available microsatellite primer kits (Merck Bioscience, USA). Individual PCR amplifications for each microsatellite were performed in programmable thermal controller (BioRad, California, USA). The PCR protocol involved a total volume of 25 μl reaction mixture (Sambrook et al., 1989) containing 35 ng of genomic DNA, 1X PCR buffer (pH 8.3), 200 μM dNTP mix, 10 pmol of each of the forward and reverse primers, 2 mM of MgCl₂ and 1 U of *Taq* (*Thermophilus aquaticus*) DNA polymerase (Merck Bioscience). The basic PCR program to amplify DNA was as follows: an initial hot start and denaturing step at 93°C for 3 min followed by 40 cycles of a 1 min denaturation at 93°C, a 1 min annealing at appropriate temperature (52 or 67°C) depending on the primer, and a 1 min primer elongation at 72°C. A final extension step at 72°C for 5 min was performed.

Gel electrophoresis

A 10 μl aliquot of the amplified microsatellite samples was combined with 2 μl of a loading buffer (0.4% bromophenol blue, 0.4% xylene cyanole and 5 ml of glycerol) and was loaded directly on 2.5% agarose gels in 0.5X TBE buffer (Sambrook et al, 1989). Electrophoresis was done for about 3 h at 60 V. Low range DNA ruler (Merck Bioscience) was used to compare the molecular weights of amplified products. Visualization of the amplified bands was done by staining with ethidium bromide for 20 min and de-staining with double distilled water for 20 min followed by trans-illumination under short wave UV light (Gel Doc. 2000, BioRad, California, USA).

Data analysis

Polymorphic products from microsatellite analyses were scored qualitatively for presence (1) and absence (0) for each marker allele-genotype combination. The data entry was done into a binary data matrix as discrete variables. Most informative primers were selected based on the extent of polymorphism. The polymorphic information content (PIC) was calculated by applying the formula given by Powell et al. (1996):

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

Where, f_{ij} is the frequency of j^{th} allele for marker i and the summation extends over n alleles. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency). Pair-wise similarity matrices based on DNA profile data were determined using Jaccard's similarity coefficient (JSC) (Sneath and Sokal, 1973). Genetic relationships among the genotypes studied were calculated using UPGMA cluster analysis and Principal Component Analysis (PCA) of the similarity matrix obtained from the proportion of shared amplification fragments. All those analyses were computed with the program NTSYSpc 2.2 (Exeter Software, Stauket, NY, USA) (Rohlf, 2002).

RESULTS AND DISCUSSION

Morphological and seed traits have the important means

of studying the taxonomy and variability among plant species. Traditional rice varieties, or landraces, have a high level of genetic heterogeneity compared to modern varieties. This genetic variability is utmost important for the sustainability of small marginal farmers, because despite the low yield capacity, these varieties /landraces present high tolerant to abiotic and biotic stresses. The phenotypic characteristics, seed morphology and their distribution of 48 traditional indigenous aromatic rice germplasm are presented in Table 2 and Figure 1. Landraces are adapted to local, small-scale, low-input environments where the plant ideotype may differ considerably from that developed for modern agricultural systems (Veteläinen et al., 1997). The evaluation of the genetic variability of accessions of landraces can provide the basic information necessary to help properly conserve these genetic resources. This will also help breeding programs to plan crosses to incorporate this variability into the genetic background of elite rice germplasms, which in turn will generate new rice cultivars. The microsatellites or SSRs are amongst the most widely used DNA marker for various purposes such as diversity, genome mapping and varietal identification (Nagaraju et al., 2002; Singh et al., 2004). The present study aimed to determine the genetic diversity of 48 traditional indigenous aromatic rice germplasm of Indian origin by using 12 SSR markers (Table 3). The results indicated a high level of genetic variation in the germplasm tested. A total of 28 different reproducible bands (alleles) ranging in size from 100bp to 220bp were amplified (Figure 2 and Table 4). The average number of bands per primer was found to be 2.33. Of the total 28 bands generated, 25 SSR bands were polymorphic whereas 1 bands generated from the SSR primer RM-44 and 2 bands in RM-154 were monomorphic. The average number of polymorphic bands per primer was 2.08. The PIC ranged from 0 to 0.74 with an average of 0.582. Pair-wise estimates of similarity matrix ranged from 0.20 to 0.92 and average Jaccard's similarity among all 48 genotypes was 0.5. The cluster analysis by using UPGMA algorithm indicated that all the 48 aromatic rice germplasm were grouped into two Clusters (I and II) at 49% similarity coefficient (Figure 3). 11 aromatic rice genotypes were represented in Cluster I whereas 37 varieties were placed in Cluster II. Cluster I was divided into two sub-clusters 'IA' and 'IB' at 56% similarity coefficient. The sub-cluster 'IA' included seven aromatic rice varieties in which 'Kaminibhog-1' and 'Kalikati-1' were most similar genotypes within sub-cluster. The genotypes included in this sub-cluster were 'Bananangemati', 'Gatia', 'Kaminibhog-1', 'Kalikati-1', 'Dhobalachi', 'Ganjeikalli' and 'Jiphulla'. On the other hand the sub-cluster 'IB' was represented by the following four aromatic rice varieties 'Bhasumatidhan' 'Basumati Bhog', 'Chatianak' and 'Pumpudibasa'. Among them 'Basumati dhan' was the most diverged one in this sub-cluster. The cluster II was further classified into two

sub-clusters 'IIA' and 'IIB'. There were 35 aromatic rice varieties included in the sub-cluster 'IIA' whereas only two aromatic rice varieties 'Dubraj' and 'Sujata' were placed in Cluster 'IIB'. Further at 68% similarity coefficient, the sub-cluster 'IIA' was divided into 3 sub-sub-clusters. Sub-sub-Cluster 'IIA₁' was represented by 7 aromatic rice germplasm among them 'Basnaparijat' was the most diverse one. In this sub-sub-cluster, the rice varieties 'Kalikati-2' and 'Karpurkranti' had shown the maximum similarity (100%). Similarly, ten aromatic rice germplasm placed in sub-sub-cluster 'IIA₂' and among them 'Sirimula' and 'Tulashiphula' exhibited the maximum similarity (100%). In Sub-sub-cluster 'IIA₃', the highest number of aromatic rice varieties (18) were placed. The aromatic rice variety 'Khoshkani' and 'Kaminibhog-2' had the maximum similarity (100%). Another pair of genotypes (Ratnasundari' and 'Tulasiphada) had also exhibited maximum similarity.

All the genotypes re-presented in this sub-cluster were similar with respect to most of the morphological characters. The PIC ranged from 0 to 0.74 with an average of 0.582. Thomson et al. (2007) analyzed 246 Indonesian accessions and 63 Indonesian improved cultivars, using 30 fluorescently-labeled micro-satellite markers and reported that a total of 394 alleles were detected at the 30 simple sequence repeat loci, with an average number of 13 alleles per locus across all accessions, and an average polymer-phism information content value of 0.66. Further, Jain et al. (2004) used 30 fluorescently labeled rice microsatellite markers and achieved a total of 235 alleles detected at the 30 SSR loci, 62 (26.4%) of which were present only in Basmati and other scented rice germplasm accessions. The number of alleles per locus ranged from 3 to 22, with an average of 7.8, PIC values ranged from 0.2 to 0.9, with an average of 0.6, and the size ranged between the smallest and the largest allele for a given microsatellite locus which varied between 3 and 68 bp. 19 SSR loci and 12 inter-SSR-PCR primers were used to assess the genetic relationships in traditional and evolved Basmati (EB) and semi dwarf non-Basmati (NB) rice varieties. A total of 70 SSR alleles and 481 inter-SSR-PCR markers were revealed in 24 varieties from the three groups (Aggarwal et al., 2002). Saini et al. (2004) evaluated the traditional Basmati, cross-bred Basmati and non-Basmati (*indica* and *japonica*) rice varieties using three DNA markers. All the three marker systems generated higher levels of polymorphism and could distinguish between all the 18 rice cultivars.

PCA analysis showed that the similarity values for related germplasm using SSRs provides greater confidence for the assessment of genetic diversity and relationships. The three-dimensional scaling of PCA analysis placed all the 48 traditional indigenous aromatic rice genotypes into four groups. Of the total 48 aromatic rice varieties, the first group contained 'Baranamgomati', 'Dhobaluchi', 'Pimpudibasa', 'Gatia', 'Jaiphulla', 'Kalikati-

Table 2. Phenotypic diversity among 48 indigenous aromatic rice genotypes.

S/N	Genotype	Days to flowering	Plant height (cm)	Panicle length (cm)	Panicle number	No of fertile grains	1000 grain weight (g)	Potential Yield (q/ha)	General characters
1	2	3	4	5	6	7	8	9	10
1	Baranamgomati	104	147.9	25.4	8	97	19.8	22.61	Tall, weak straw, Photo-sensitive, straw coloured hull, MB grains and white kernel
2	Basnasapuri	104	145.2	25.5	11	173	11.0	25.07	Tall, weak straw, Photo-sensitive, straw coloured hull, SS grains and white kernel
3	Basnaparijat	104	117.6	24.3	10	147	11.1	22.22	Tall, weak straw, Photo-sensitive, straw coloured hull, SS grains and white kernel
4	Basumati-1	100	103.3	24.1	9	62	16.9	23.95	Tall, weak straw, Photo-sensitive, straw coloured hull, SS grains and white kernel
5	Basmatidhan	91	122.4	22.3	6	116	12.8	18.62	Tall, weak straw, Photo-sensitive, straw coloured hull, SB grains and white kernel
6	Basumati Bhog	100	129.8	22.8	7	195	13.3	27.36	Tall, weak straw, Photo-sensitive, brown coloured hull, furrows on the straw, Apiculus and empty glumes purple pigmented, SB grains and white kernel
7	Chatianaki	99	140.7	29.0	7	123	12.5	19.59	Tall, weak straw, Photo-sensitive, black coloured hull, furrows on the straw, Apiculus and empty glumes purple pigmented, SB grains and white kernel
8	Dhobaluchi	91	103.9	18.8	7	123	23.4	27.46	Tall, weak straw, Photo-sensitive, straw coloured hull, MB grains and white kernel
9	Krishnabhoga	102	128.6	19.1	6	128	22.1	25.22	Tall, weak straw, photo-sensitive, Hull colour reddish brown furrows on straw, Apiculus and empty glumes purple pigmented, SB grains and white kernel
10	Manasi-2	101	115.4	24.6	9	117	13.2	23.48	Tall, weak straw, photo-sensitive, Hull colour brown furrows on straw, Apiculus and empty glumes purple pigmented, SB grains and white kernel
11	Nuakalajeera	105	136.1	27.2	7	159	11.0	20.78	Tall, weak straw, photo sensitive, Hull colour black, SB grains and white kernel and apiculus pigmented
12	Nuadhusura	104	125.3	25.5	8	110	15.9	20.72	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel
13	Pimpudibasa	100	124.5	26.4	6	160	13.3	18.57	Tall, weak straw, photo-sensitive, hull colour deep black furrows on straw, apiculus and empty glumes purple pigmented, SB grains and white kernel
14	Ratnasundari	104	126.3	19.3	10	118	14.5	19.64	Tall, weak straw, photo-sensitive, hull colour reddish furrows on straw , apiculus purple pigmented, SS grains and white kernel
15	Sujata	96	115.3	24.7	9	140	14.1	26.32	Tall, weak straw, photo-sensitive, straw coloured hull, MS grains and white kernel

MB, Medium bold; SB- slender bold.

Table 2. Contd.

S/N	Genotypes	Days to flowering	Plant Height (cm)	Panicle length (cm)	Panicle number	Nmber of fertile grains	1000 grain weight (g)	Potential Yield (q/ha)	General characters
1	2	3	4	5	6	7	8	9	10
16	Srimula	105	125.9	25.2	8	131	11.1	21.59	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel
17	Thakurbhog	106	127.4	28.8	10	145	12.9	28.12	Tall, weak straw, photo-sensitive, hull colour black, apiculus purple pigmented, SB grains and white kernel
18	Tulasiphada	102	123.4	27.3	6	126	13.8	24.70	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel
19	Sheetabhog	103	120.6	23.6	8	145	10.7	19.13	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel
20	Gopalabhoga	102	121.5	24.6	7	125	11.5	18.57	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel
21	Tulasiphulla	98	110.8	23.2	9	108	13.7	10.49	Tall, weak straw, photo-sensitive, hull colour reddish brown on straw, apiculus pigmented, SB grains and white kernel
22	Bhasumati (P)	97	126.3	24.4	8	125	12.4	23.54	Tall, weak straw, Photo-sensitive, straw coloured hull, SS grains and white kernel
23	Saragadhulli	103	129.5	25.6	10	132	11.8	19.23	Tall, weak straw, photo-sensitive, hull colour reddish brown furrows on straw , apiculus and empty glumes purple pigmented, SB grains and white kernel
24	Mugajai	105	126.4	26.4	6	153	17.5	22.45	Tall, weak straw, photo-sensitive, straw coloured hull, Apiculus purple pigmented, SB grains and white kernel
25	Dholabankoi	102	127.1	27.1	9	169	12.4	29.42	Tall, weak straw, Photo-sensitive, brown coloured hull, furrows on the straw, Apiculus and empty glumes purple pigmented, SB grains and white kernels
26	Dubraj	103	125.4	28.4	8	152	14.5	26.37	Tall, weak straw, Photo-sensitive, straw coloured hull, MS grains and white kernel and awned
27	Dulhabhoga	100	125.2	23.0	7	141	18.9	17.65	Tall, weak straw, Photo-sensitive, brown coloured hull, furrows on the straw, Apiculus and empty glumes purple pigmented, SB grains and white kernels
28	Dangarabasamati	101	126.0	22.8	7	135	15.6	19.20	Tall, weak straw, Photo-sensitive, straw coloured hull, SS grains and white kernel
29	Gangaballi	97	125.4	25.0	9	139	11.6	24.48	Tall, weak straw, Photo-sensitive, straw coloured hull, SS grains and white kernel
30	Ganjam local-1	98	123.0	23.9	8	137	11.8	19.20	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel

Table 2. Contd.

S/N	Genotypes	Days to flowering	Plant Height (cm)	Panicle length (cm)	Panicle number	Number of fertile grains	1000 grain weight (g)	Potential Yield (q/ha)	General characters
1	2	3	4	5	6	7	8	9	10
31	Ganjam local-2	105	130.0	24.5	7	128	13.9	27.29	Tall, weak straw, photo-sensitive, black coloured hull, SB grains and white kernel and apiculus pigmented
32	Ganjeikalli	95	127.1	19.4	10	130	18.3	25.42	intermediate height , stiff straw, Photo-sensitive, straw, golden coloured hull, SB grains and dull white kernel
33	Gatia	107	136.2	23.2	8	135	18.0	24.37	Tall, weak straw, Photo-sensitive, straw, golden coloured hull, apiculus pigmented, SB grains and dull white kernel
34	Heerakani	98	117.6	25.2	10	175	11.0	23.16	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel
35	Jalaka-1	104	107.4	24.7	6	112	14.2	22.81	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel
36	Jalaka-2	103	115.4	24.5	10	136	11.6	18.71	Tall, weak straw, photo-sensitive, straw coloured hull, SS grains and white kernel
37	Jaiphulla	102	105.0	20.6	6	107	19.2	25.68	Tall, weak straw, photo-sensitive, straw coloured hull, MS grains and white kernel
38	Kalajeera	104	129.9	25.6	8	123	14.4	25.55	Tall, weak straw, photo-sensitive, straw coloured hull, MS grains and white kernel
39	Kalajauvan	101	140.3	29.8	4	143	13.5	18.60	Tall, weak straw, photo-sensitive, Hull coloured brown furrows on straw, SB grains and white kernel
40	Khosakani	104	127.8	27.4	7	174	13.9	28.41	Tall, weak straw, photo-sensitive, Black coloured hull, SB grains, white kernel and apiculus pigmented
41	Kaminibhoga-1	96	126.4	20.3	6	163	14.6	20.31	Tall, weak straw, photo-sensitive, Straw coloured hull, SS grains and whit kernel
42	Kaminibhoga-2	103	129.0	25.2	7	145	11.9	19.77	Tall, weak straw, photo-sensitive, Straw coloured hull, SS grains and whit kernel
43	Kalikati-2	105	141.1	27.7	13	174	12.3	24.07	Tall, weak straw, photo-sensitive, Red colour hull, SS grains and white kernel
44	Kalikati-1	101	133.1	24.9	7	164	10.9	21.39	Tall, weak straw, photo-sensitive, Hull colour reddish brown furrows on straw, Apiculus and empty glumes purple pigmented, SB grains and white kernel
45	Kanakachampa	98	135.6	24.5	7	146	12.1	18.62	Tall, weak straw, photo-sensitive, Straw coloured hull, SS grains and white kernel
46	Kusumabhoga	97	128.2	22.1	12	138	13.6	19.42	Tall, weak straw, photo-sensitive, Hull colour brown furrows on straw, Apiculous and empty glumes purple pigmented, SB grains and white kernel
47	Karpurakranti	102	130.9	24.5	7	88	13.6	17.65	Tall, weak straw, photo-sensitive, Hull colour black furrows on straw, Apiculus and empty glumes purple pigmented, SB grains and white kernel

Table 2. Contd.

48	Manasi-1	100	122.3	22.3	6	72	15.1	19.67	Tall, weak straw, photo-sensitive, Hull colour brown furrows on straw, Apiculus and empty glumes purple pigmented, SB grains and white kernel
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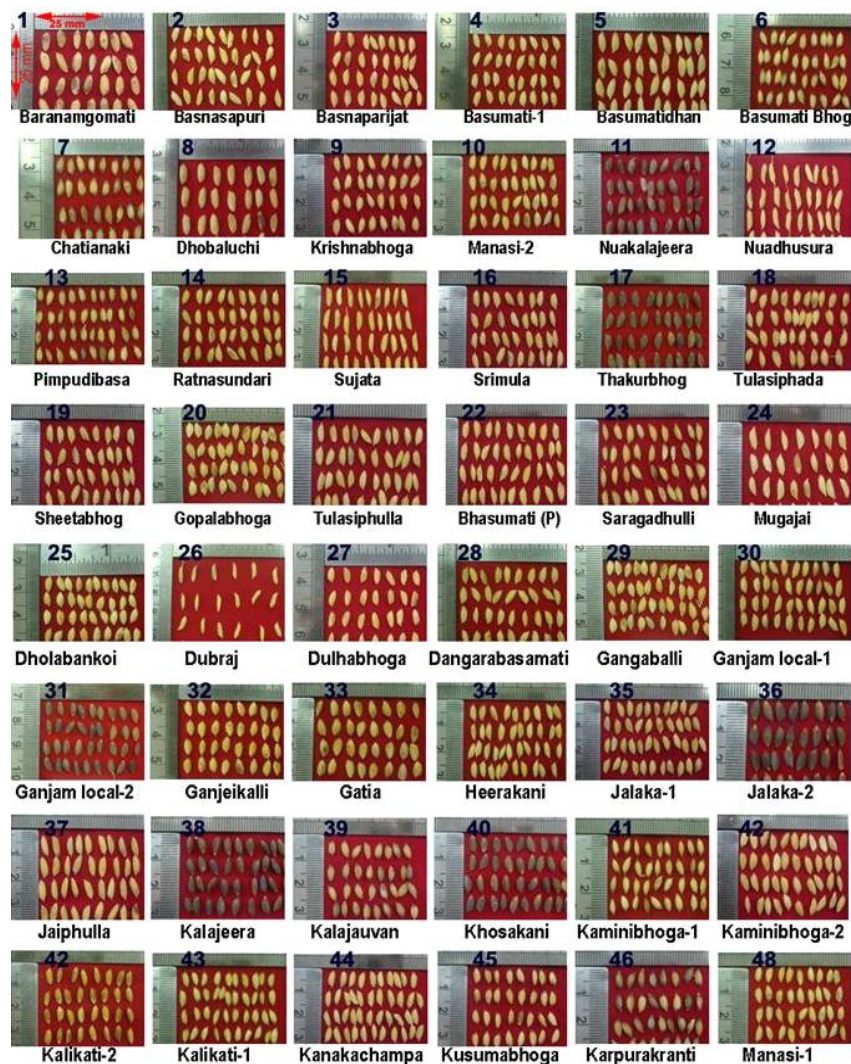


Figure 1. Grain morphology of 48 indigenous aromatic rice genotypes.

Table 3. Details of the SSR primers used for genetic analysis.

Primer	Sequence (5'-3')	Tm*	Ta*	Chromosome number of rice genome
RM9	F-5 "GGTGCCATTGTCGTCCTC 3"	64.4°C	62°C	1
	R-5 "ACGGCCCTCATCACCTTC 3"	65.2°C	62°C	1
RM154	F-5 "ACCTCTCCGCGCTCGCCTCCTC 3"	64.4°C	62°C	1
	R-5 "CTCCTCCTCCTGCGACCGCTCC 3"	65.2°C	62°C	1
RM218	F-5 "TGGTCAAACCAAGGTCCTTC 3"	63.8°C	55°C	3
	R-5 "GACATACATTCTACCCCGG 3"	62.9°C	55°C	3
RM241	F-5 "GAGCCAAATAAGATCGCTGA 3"	61.8°C	52°C	4
	R-5 "TGCAAGCAGCAGATTTAGTG 3"	61.7°C	52°C	4
RM249	F-5 "GGCGTAAAGGTTTTGCATGT 3"	63.6°C	52°C	5
	R-5 "ATGATGCCATGAAGGTCAGC 3"	65.0°C	52°C	5
RM30	F-5 "GGTTAGGCATCGTCACGG 3"	64.2°C	55°C	6
	R-5 "TCACCTCACACACGACACG 3"	68.7°C	55°C	6
RM234	F-5 "ACAGTATCCAAGGCCCTGG 3"	63.8°C	55°C	7
	R-5 "CACGTGAGACAAAGACGGAG 3"	63.5°C	55°C	7
RM228	F-5 "CTGGCCATTAGTCCTTGG 3"	60.6°C	55°C	10
	R-5 "GCTTGCGGCTCTGCTTAC 3"	63.8°C	55°C	10
RM21	F-5 "ACAGTATTCGGTAGGCACGG 3"	63.7°C	55°C	11
	R-5 "GCTCCATGAGGGTGGTAGAG 3"	63.6°C	55°C	11
RM20	F-5 "ATCTTGTCCCTGCAGGTCAT 3"	63.5°C	55°C	12
	R-5 "GAAACAGAGGCACATTCATTG 3"	63.3°C	55°C	12
RM44	F-5 "ACGGGCAATCCGAACAACC 3"	63.3°C	55°C	8
	R-5 "TCGGGAAAACCTACCCTACC 3"	63.3°C	55°C	8
RM 444	F-5 "GCTCCACCTGCTTAAGCATC 3"	63.8°C	55°C	3
	R-5 "TGAAGACCATGTTCTGCAGG 3"	62.9°C	55°C	3

Tm and Ta represent the melting temperature and annealing temperature of the primers, respectively.

2', 'Kaminibhoga-2'. The second group contained 'Ganjeikalli' and the third group contained 'Dubraj', 'Sujata,' 'Chatianaki', 'Basumati Bhog', 'Basmatidhan', 'Basnaparijat', and remaining varieties were placed in IV group in the co-ordinate axis (Figure 4). The phenotypic characteristics had positive correlation with polymorphic information content. The PIC ranged from 0.07 to 0.74 with an average of 0.582 (Table 4). The molecular data were generally correlated with variation at the morphological level in the crop plants and therefore provide good guidance on the distribution of useful variation as well as on the existence of co-adapted gene complexes (Hawtin et al., 1997). Despite the fact that the SSR markers used in our study are considered neutral, the genomic fingerprinting profile of each germplasm does not vary from one environment to another, reflecting the capacity of an individual plant or population to adapt (Virk et al., 1996). This direct association between the fingerprint of an accession and the phenotypic response to a target environment is caused by linkage disequilibrium, which in rice is mainly due to autogamous reproduction (Ford-Lloyd et al., 1997). Neutral markers can be used to establish the evolutionary past of varietal groups and to account for pre-selection of the germplasm to be

used in breeding programs (Glaszmann et al., 1996; Brondani et al., 2006). One of the implications of SSR analysis for landrace conservation is that the genotyping of landraces can detect differences that cannot be detected by traditional methods of morphological characterization used routinely in gene banks. The knowledge of within genotypic variability is important for conservation purposes, because it is possible to determine the most genetically variable accessions which would demand an additional effort of sampling a higher quantity of seeds in order to preserve this genetic variability and prevent genetic drift during routine periodic germplasm multiplication. The analysis of individual plants of an accession is also relevant for breeding purposes, since homozygous plants can be selected and used as genitors in crosses with elite rice genotypes. In addition, marker-based identification and differentiation of aromatic rice could be helpful to preserve the integrity of the high quality rice varieties to benefit farmers, breeders and consumers communities.

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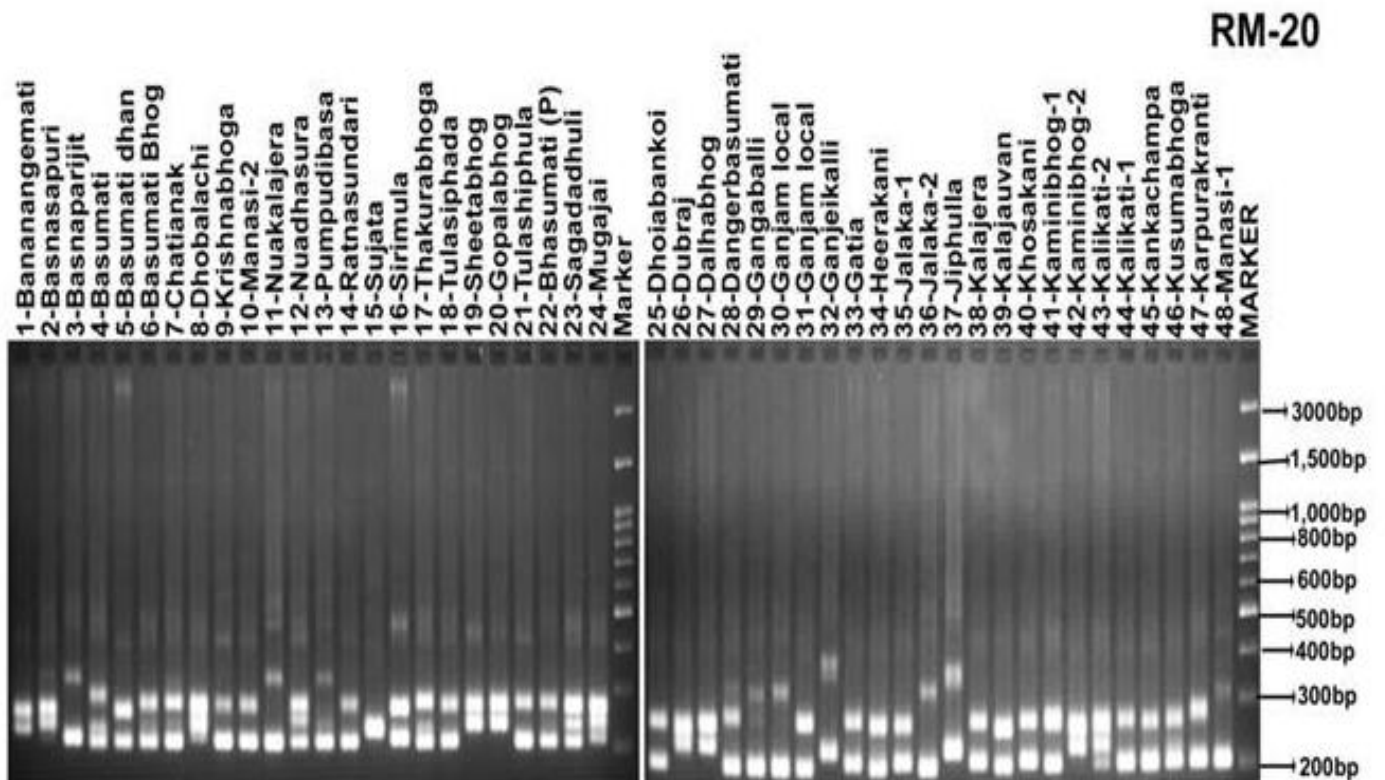
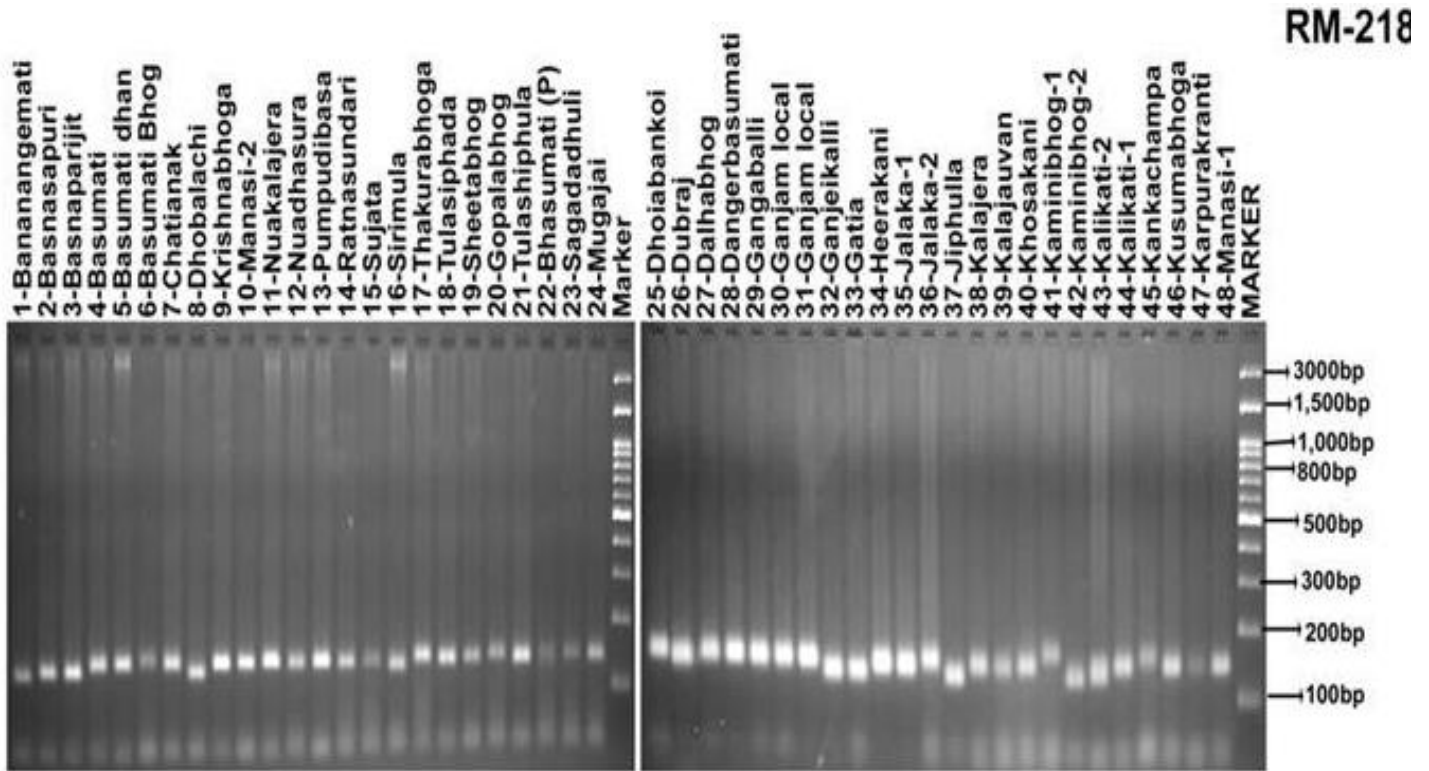


Figure 2. DNA amplification profile of 48 aromatic rice genotypes using SSR markers.

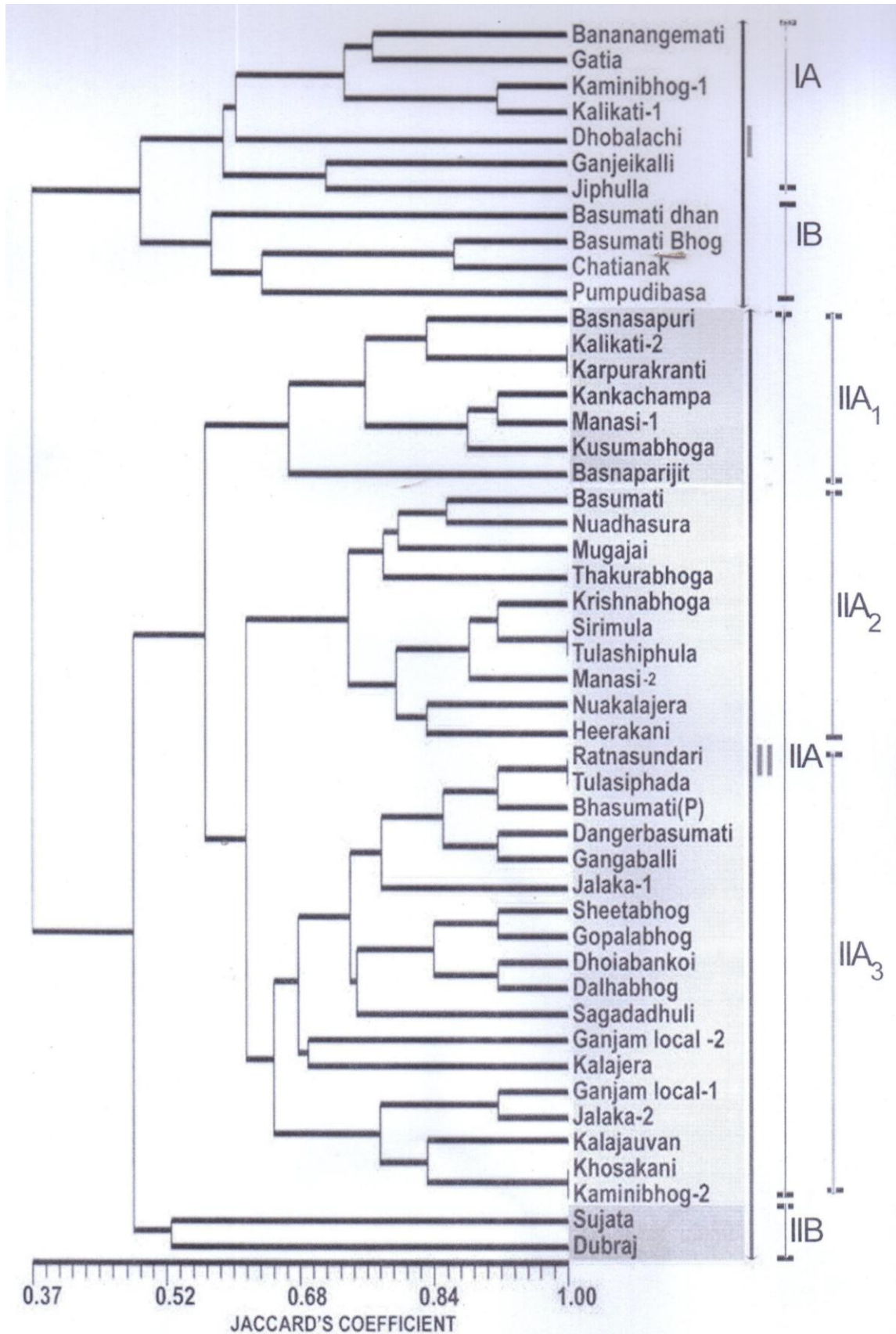


Figure 3. Cluster analysis of 48 aromatic rice genotypes on the basis of similarity index.

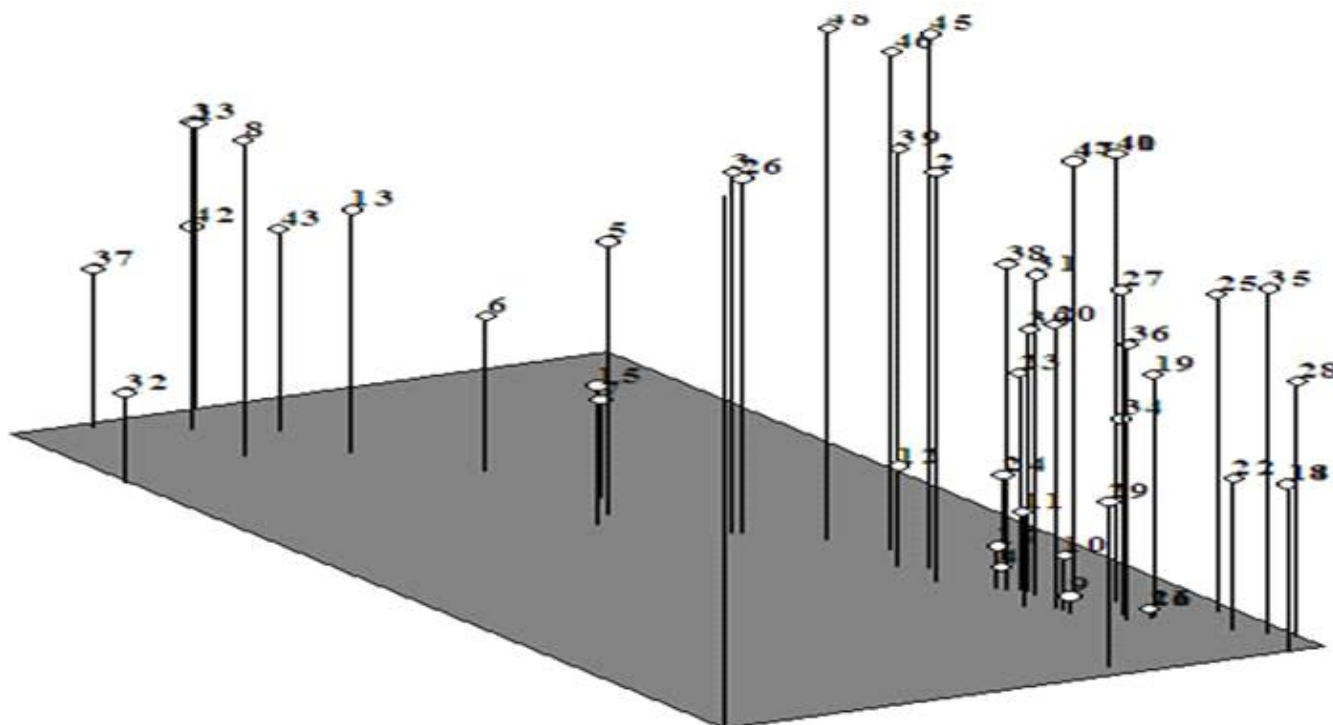


Figure 4. 3D PCA analysis of 48 aromatic rice genotypes on the basis of similarity index.

Table 4. DNA profile and polymorphism generated in 48 Aromatic rice genotypes using 12 SSR primers.

Chromosome	Position (cM)	Primer code	Number of alleles	Number of polymorphic alleles	Percentage polymorphism (bp)	Size Range(bp)	Average PIC value
1	92.4	RM 9	2	2	100	100- 150	0.74
2	4.8	RM154	4	2	50	100- 220	0.39
3	67.8	RM218	2	2	100	100-120	0.74
4	106.2	RM241	2	2	100	150-180	0.69
5	55.6	RM249	2	2	100	120-150	0.53
6	125.4	RM30	2	2	100	100-110	0.74
7	88.2	RM234	2	2	100	140-180	0.59
10	130.3	RM228	2	2	100	100-150	0.49
11	85.7	RM21	2	2	100	110-190	0.65
12	3.2	RM20	5	5	100	100-210	0.71
8	60.9	RM44	1	0	0	130	0
9	3.3	RM444	2	2	100	130-180	0.71
		Total	28	25	-	-	6.99
		Mean	2.33	2.08	-	-	0.582

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