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Full Length Research Paper

Assessment of diversity amongst a set of aromatic rice genotypes from India

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Both the level of genetic diversity and the number of rice genotypes preserved in rice germplasm banks are high but apart from the basmati the rest of the indigenous aromatic genotypes in India have received little attention. Hence, there is an urgent need to catalogue, characterize and conserve the non-basmati indigenous aromatic rice genotypes, which are inextricably integrated with religious and social ceremonies, rituals and traditional knowledge. In addition, these aromatic genotypes are vital genetic resources for agronomic and quality traits. This study analyses the diversity among 26 indigenous non-basmati aromatic rice genotypes, six basmati and 9 HYV; both morphologically using 12 grain and kernel traits and genetically using 23 previously mapped SSR markers. High genetic diversity was observed for the grain and kernel dimension and quality traits, in the indigenous non-basmati aromatic rice genotypes through D^2 analysis. The polymerase chain reaction (PCR) profile obtained from 23 SSR markers generated 172 alleles including 28 rare alleles and 9 null alleles. The ensuing dendrogram obtained from the SSR profiles clustered the basmati rice and the indigenous non-basmati aromatic rice genotypes separately.

Key words: Aromatic rice, genetic diversity, SSR polymorphism.

INTRODUCTION

Grown under diverse eco-geographical conditions in various tropical and subtropical countries, rice, (*Oryza sativa)* (2n = 24) belonging to the family, Graminae and subfamily, Oryzoidea is an ideal model plant for the study of grass genetics and genome organization due to its relatively small genome size of 430 Mb (Causse et al., 1994). Although, the level of phenotypic and genetic polymorphism of the aromatic rice genotypes stored in various rice germplasm banks around the world are high (Byerlee, 1996) but apart from the basmati genotypes the rest of the indigenous non-basmati aromatic rice have received little attention (McCouch et al., 1998; Tanksley, and Nelson 1996; Wang et al., 1990). Indigenous rice genotypes available in different countries are endowed with tremendous genetic variability and are vital genetic resources for biotic and abiotic stress resistance/ tolerance, reduction of growth duration and improved nutritional characteristics (Deb, 2000; Bhagwat et al., 2008; Agnihotri and Palni, 2007).

Each state of India has its own special aromatic rice which has been maintained by a small group of farmers mainly for their individual consumption and for sustaining certain religious rituals and social ceremonies (Bhagwat et al., 2008; Agnihotri and Palni, 2007). 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; Maxted and Kell,

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2009). In addition, some genotypes are very specific to certain location and loose their potential with change in agro-climate. For example Tulaipanji, a genotype originally cultivated in the cooler Northern districts of the state of West Bengal, India; looses its aroma when cultivated in the warmer southern districts (Deb, 2000). Hence, there is an urgent need to catalogue, characterize and conserve the non-basmati indigenous aromatic rice genotypes, which are inextricably integrated with culture and traditional knowledge of the nation. With this prerogative in mind, this study analyses the diversity among a set of 26 indigenous aromatic genotypes from the state of West Bengal, India and compares their diversity with 6 basmati genotypes and 9 check rice genotypes. The 9 check genotypes include 3 high yielding varieties, 4 international check varieties and 2 table rice genotypes from West Bengal. The total set of rice genotypes chosen for this study is 41, all of which were examined for twelve grain and kernel traits and genetic polymorphism using 23 previously mapped SSR markers.

The grain kernel traits include dimension traits like grain and kernel length and breadth and length/breadth ratio (a measure of slenderness); cooked kernel elongation, 100 grain weight and quality traits like aroma, alkali spreading value and amylose content. The length and slenderness of rice grains and kernels determine the price of aromatic rice in national and international markets. Longer cooked kernel is a desired characteristic of the prized basmati rice. Aroma of rice has mainly been attributed to 2-acetyl-1-pyrroline which is present in all rice, but is present in significantly higher (10 times) concentrations in the aromatic cultivars (Buttery et al., 1983). A comparison of aroma was made among the indigenous aromatic and the basmati varieties. The trait alkali spreading value is a measure of gelling temperature (GT), which refers to the cooking temperature at which water is absorbed and the endosperm starch granule swell irreversibly with simultaneous loss of crystalline structure. It also provides a simple means of classifying rice into high, intermediate and low gelatinization temperature types (Little et al., 1958). The trait amylose content (measured in percentage) is directly proportional to stickiness of cooked rice kernels (Jennings et al., 1979). In addition to the grain and kernel traits, the genetic diversity of the chosen 41 genotypes was assessed using SSR markers. Simple sequence repeat (SSR) markers or microsatellites are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions (Giovannoni et al., 1991), (McCouch et al., 1997). Phenotypic and protein or isozyme marker polymorphisms are influenced by environmental effect whereas Simple Sequence Repeat (SSR) markers are more popular in rice because they are highly informative, mostly mono locus, co-dominant, easily analyzed and cost effective (Chambers and Avoy, 2000). Compared to RFLPs, microsatellite markers detect a significantly higher degree of polymorphism in rice (Wu and Tanksley, 1993; Yang et al., 1994), and are especially suitable for evaluating genetic diversity among closely related rice cultivars (Akagi et al., 1997). SSR generated DNA profiles are used for identification of germplasms and their protection under the Trade Related Intellectual Property Rights (TRIPS) of the World Trade Organization (WTO), (Bhat, 2001).

In the Indian subcontinent, previous works on genetic diversity analysis using SSR polymorphism focused mainly on the basmati genotypes. Nagaraju et al. (2002) used fluorescence-based inter simple sequence repeat-PCR (ISSR-PCR) and simple sequence repeat (SSR) markers for genetic analysis of 6 traditional basmati (TB), 11 evolved basmati (EB) 8 and semi-dwarf non-basmati aromatic rice genotypes. A total of 70 SSR alleles and 481 ISSR-PCR markers were identified in the 25 varieties from the three groups.

The TB varieties showed the lowest genetic diversity, whereas the EB varieties show high genetic diversity by both the marker assays. In a similar study using fluorescent-amplification fragment length polymorphism (f-AFLP), Aggarwal et al. (2002) analyzed the genetic diversity and interrelationships amongst 33 rice genotypes consisting of the traditional basmati, improved basmati-like genotypes developed in India and in other countries, American long grain rice and a few non-aromatic rice lines. Jain et al. (2003) analysed some commercially important basmati rice varieties using 15 SSR mapped markers (Temnykh et al., 2000), including an SSR marker (SCU-rice-SSR1) developed for the RG28 locus (Garland et al., 2000). Saini et al. (2004) evaluated the genetic diversity and patterns of relationships among the 18 rice genotypes representative of the traditional basmati and cross-bred basmati rice varieties using AFLP, ISSR and SSR markers. Genetic diversity analysis of basmati rice has also been undertaken recently Archak et al. (2007), Rashid et al. (2009), Rekha (2011) and Singh et al. (2011).

Comparatively non-basmati indigenous aromatic rice genotypes have received little attention and there has been lesser number of DNA fingerprinting studies on them. Prashanth et al. (2002) estimated the genetic diversity among 49 Indian rice accessions including 29 landraces from Jeypore, 12 modern cultivars, and 8 traditional cultivars from Tamil Nadu using AFLP markers. Joshi and Behera (2006) used twelve microsatellite markers, one from each chromosome of rice for evaluating the genetic diversity of 38 traditional indigenous non-Basmati aromatic rice cultivars which included 7 genotypes from West Bengal. Jain et al. (2004) evaluated the genetic relationships among 22 Indian aromatic and quality rice germplasm from different parts of India, 30 basmati and 17 indica and japonica varieties using 30 fluorescently labeled rice microsatellite markers wherein a total of 235 alleles were detected.

Table 1. Names, sources and the category of the collected rice genotypes.

Sr. no.	Genotype name	Source	C*	Sr. no.	Genotype name	Source	C*
1	Badshahbhog	RRS, Chinsurah	IA	22	Radhunipgol 1	RRS, Sekhampur	IA
2	Chinikamini 1	RRS, Chinsurah	IA	23	Radhunipgol 2	RRS, Chinsurah	IA
3	Chinikamini 2	RRS, Chinsurah	IA	24	Tulaipanji	RRS, Sekhampur	IA
4	Danaguri	RRS, Chinsurah	IA	25	Tulsibhog	RRS, Sekhampur	IA
5	Gobindobhog 1	RRS, Chinsurah	IA	26	Tulsimanjari	ATC, Fulia	IA
6	Gobindobhog 2	ATC, Fulia	IA	27	Basmati 370	ATC, Fulia	TB
7	Gopalbhog	ATC, Kashipur	IA	28	Karnal local	ATC, Fulia	TB
8	Kalogobindobhog	ATC, Fulia	IA	29	Mahisugandha	RRS, Chinsurah	EB
9	Kalojira	ATC, Fulia	IA	30	Pakistani Basmati	ATC, Fulia	TB
10	Kalonunia	ATC, Kashipur	IA	31	Pusa Basmati 1	RRS, Chinsurah	EB
11	Kanakchur	ATC, Kashipur	IA	32	Taraori Basmati	ATC, Fulia	TB
12	Kaminibhog	ATC, Kashipur	IA	33	IR-8	RRS, Chinsurah	ICV
13	Kataribhog	RRS, Chinsurah	IA	34	IR-36	RRS, Chinsurah	ICV
14	Khasdhan	ATC, Kashipur	IA	35	IR-64	RRS, Chinsurah	ICV
15	Lilabati	ATC, Fulia	IA	36	Khitish	RRS, Chinsurah	HYV
16	Mohanbhog	ATC, Fulia	IA	37	Satabdi	RRS, Chinsurah	HYV
17	Narayan bhog	ATC, Fulia	IA	38	Lal swarna	ATC, Kashipur	HYV
18	Narayan purna	ATC, Fulia	IA	39	TN-1	RRS, Chinsurah	ICV
19	NC 324	RRS, Chinsurah	IA	40	Chamarmani	ATC, Fulia	TR
20	NC 365	RRS, Sekhampur	IA	41	Dudherswar	ATC, Kashipur	TR
21	Radhatilak	ATC, Fulia	IA				

C* - category of rice, IA- Indigenous non-basmati aromatic rice from West Bengal, TB - Traditional Basmati, EB - Evolved Basmati, HYV - High Yielding Varieties, ICV - International Check Varieties, TR – Table rice, RRS – Rice research station, ATC – Agricultural training centre.

MATERIALS AND METHODS

Plant materials

A total of 41 rice genotypes were collected from various rice research stations of India. The names and category of the rice genotypes and the names of the corresponding sources are given in Table 1.

Measurement of grains and kernels traits

A total of twelve traits were measured during this study. They were grain length (GL), grain breadth (GB), grain length/breadth (G L/B), 100 grain weight (100 GW), kernel length (KL), kernel breadth (KB), kernel length/breadth (K L/B), kernel length after cooking (KLAC), kernel elongation ratio (KER), alkali spreading value (ASV) (in a scale of 1 to 7 according to the method of Little et al., 1958), amylose percentage (AMY %) and aroma (ARO) (in a scale of 0 to 3 according to Sood and Siddiq,1978).

ANOVA and D^2 analysis of grain and kernel trait data

The data for the grain kernel traits of the 41 Indian genotypes were analyzed statistically for difference in means through ANOVA using the software SPSS 10.0. D^2 analysis (Mahalanobis genetic divergence analysis) was done for the quantitative and qualitative data using the software Indostat. The genetic divergence values (D^2 values) between all possible pairs of the genotypes were arranged into a matrix and a dendrogram of the data were obtained using the UPGMA method with the help of the software SPSS 10.0.

Isolation of rice genomic DNA and PCR amplification

Three days old rice seedlings were used for genomic DNA isolation according to the method of Walbot (1988). PCR amplification of this DNA was done with twenty three pairs of SSR markers. The name, motif, chromosomal location and annealing temperature of those markers are given in Table 2. DNA amplification was carried out in 25 µl volumes using 200 µl thin-walled PCR tubes (Axygen, USA) in a MJR thermal cycler. Each reaction mixture contained 1 µl of genomic DNA (100 ng), 0.5 µl of each of the two primers (at a concentration of 10 pmole/µl), 2.5 µl of a 10X PCR buffer, 0.75 µl of a 50 mM $MgCl_2$ solution, 0.25 µl of a 2.5 mM dNTP mixture, 0.2 µl (1 unit) of Taq DNA polymerase (conc. 5 unit/µl) and 19.3 µl of PCR-grade water. The temperature profile used for PCR amplification comprised 97°C for 5 min, 55 to 60°C (as necessary in accordance to Table 2) for 2 min; followed by 35 cycles of 1 min at 95°C, 1 min at 55 to 60°C and 2 min at 72°C. The final extension was at 72°C for 10 min.

Polyacrylamide gel electrophoresis

The PCR products were resolved by native polyacrylamide gel electrophoresis (PAGE) following the protocol given by Sambrook et al. (1989) in a 6% gel in vertical electrophoresis tank (gel size of 16 × 14 cm, Biotech, India) with Tris-Acetate-EDTA buffer at 150 V supplied by a power pack. The gel, after electrophoresis, was stained with ethidium bromide (5 µg of EtBr in 200 ml of Tris-Borate-EDTA buffer) washed thoroughly double distilled water and photographed using a Gel Documentation System (Bio-Rad, USA). The length of the amplified DNA bands (microsatellite alleles) from

Table 2. Name, motif, chromosomal location and annealing temperature rice microsatellite (RM) or SSR markers used for this study.

Sr. no.	Locus	Motif	Chr*	T**	Sr. no.	Locus	Motif	Chr*	T**
1	RM 42	(GA)6	8	65	13	RM 250	(CT)17	2	60
2	RM 44	(GA)16	8	55	14	RM 251	(CT)29	3	55
3	RM72	(TAT)5C(ATT)15	8	55	15	RM 282	(GA)15	3	59
4	RM 80	(CTT)20	8	65	16	RM 284	(GA)8	8	55
5	RM 112	(GAA)5	2	55	17	RM 310	(GT)19	8	55
6	RM 149	(AT)10	8	59	18	RM 337	(CTT)4-19-(CTT)8	8	59
7	RM 152	(GGC)10	8	60	19	RM 339	(CTT)8CCT9CCT)5	8	59
8	RM 182	(AT)16	7	59	20	RM 341	(CTT)20	2	55
9	RM207	(GA)25	2	65	21	RM 505	(CT)12	7	55
10	RM 210	(GA)23	8	55	22	RM 530	(GA)23	2	59
11	RM 218	(GA)24	3	55	23	RM 569	(CT)16	3	59
12	RM 223	(GA)25	8	55					

Chr* – chromosome on which marker is located, **T**** – annealing temperature.

the different rice genotypes was determined with reference to the 100bp DNA ladder (SibEnzyme) using the software Quantity One (Bio-Rad, USA).

The different alleles amplified form the genomic DNA of the 41 rice genotypes were identified on the basis of their size, or length in base pairs (bp). A 1/0 matrix for the presence and absence of all the alleles in the genotypes were produced for each of the 23 SSR markers. The resultant binary matrix was subjected to cluster analysis using software SPSS 10. Dice similarity coeffi-cient was employed to compute pair-wise genetic similarity. The corresponding dendogram (cluster diagram) was constructed by applying un-weighted pair group method with arithmetic average (UPGMA) using the software SPSS 10.0. Polymorphism information content, or PIC value, for the SSLP markers was calculated by the simple formula:

$PIC_i = 1 - \Sigma_{i=1}^{n} P^2_{ij}$,

Given by Anderson et al. (1993), where P_{ij} is the frequency of the j^{th} allele for the i^{th}, SSR marker.

RESULTS

Measurements of grain and kernel traits

The measurements of the grain and kernel traits for the 41 Indian rice genotypes and their mean values are given in Table 3. From the table it can be inferred that Pusa basmati 1, an evolved basmati has the highest value for six of the traits - grain length/breadth ratio, kernel length/breadth ratio, kennel length after cooking, kernel elongation ratio, alkali spreading value and aroma. Kaminibhog, an indigenous aromatic landrace from the state of West Bengal, India has the lowest value for three traits - grain length, grain length/breadth ratio and kernel length/breadth ratio. Thus the grains and kernels of Kaminibhog are short and rounded and for Pusa basmati 1 they are long and slender.

Figure1 show a comparison between the minimum and the maximum values of grain length, kernel length, and kernel length after cooking, grain breadth and kernel breadth among indigenous non-basmati aromatic genotypes, basmati genotypes and high yielding varieties. In the graph, each category of rice is represented in bars different colour. For all the traits, bar number 1 and 2 represent the minimum and maximum values for the trait, respectively, for indigenous non-basmati aromatic rice genotypes, bars 3 and 4 represent the same for the basmati group of genotypes, and bar numbers 5 and 6 represent the same for the HYVs. It can be observed from the graph that the maximum values of grain and kernel length is highest for the basmati genotype than indigenous non-basmati aromatic rice and the high yielding varieties. In case of the indigenous aromatic genotypes the maximum values for the traits mentioned above is almost the same as the minimum value of the basmati rice for the same traits. For the traits grain and kernel breadth the maximum values for indigenous non-basmati aromatic rice is more than that of the basmati genotype. Hence a longer grain and kernel length coupled with shorter grain and kernel breadth make the basmati more slender than the indigenous non-basmati aromatic rice.

Figure 2 shows a comparison of the grain length/ breadth ratio, kernel length/breadth ratio (a measure of slenderness) and kernel elongation ratio among indigenous non- basmati aromatic genotypes, basmati genotypes and high yielding varieties. The layout of data in this graph is the same as in Figure 1. It can be observed from the graph that the minimum and maximum values of G L/B and K L/B ratio is higher for the Basmati rice than indigenous non-basmati aromatic rice and the high yielding varieties. So it can be concluded from this observation that the grains and kernels of basmati rice are more slender. However the maximum values of kernel elongation ratio for indigenous aromatic genotypes

Table 3. Mean values of twelve grain and kernel traits of the 41 rice genotypes.

No.	Genotypes	GL	GB	G-L/B	100 GW	KL	KB	K-L/B	KLAC	KER	ASV	AMY %	ARO
1	Badshahbhog	6.78	2.33	2.91	1.12	4.78	1.93	2.48	9.32	1.95	2.67	19.31	1.33
2	Gobindobhog	6.13	2.18	2.81	1.03	4.52	1.85	2.45	8.4	1.86	2.67	15.39	3
3	Gobindobhog	6.38	2.18	2.93	1.13	4.61	1.85	2.49	8.17	1.77	2.67	15.33	2
4	Gopalbhog	6.51	2.16	3.02	1.06	4.65	1.87	2.48	8.68	1.87	3	21.43	1.33
5	Kaminibhog	5.82	2.75	2.12	1.46	4.33	2.45	1.77	7.72	1.53	3	21.03	1.33
6	Katarihog	7.99	2.23	3.58	1.41	5.64	1.89	2.99	10.6	1.88	3	15.42	1.33
7	Mohanbhog	5.86	2.25	2.6	1.11	4.18	1.86	2.25	8.17	1.96	2.67	16.62	1.67
8	Tulsibhog	6.75	2.21	3.06	0.95	4.32	1.87	2.31	7.85	1.82	2.67	20.47	1
9	Narayan bhog	8.94	2.24	4	1.4	6.88	1.97	3.49	11.3	1.65	3	17.49	1
10	Kalogobindobhog	8.09	2.01	4.03	0.97	5.74	1.88	3.05	10.1	1.76	2.67	16.86	1
11	Chinikamini 1	5.87	2.7	2.17	1.22	4.07	2.25	1.81	8.17	2.01	3.67	19.5	2.67
12	Chinikamini 2	8.63	2.49	3.47	1.87	6.07	1.94	3.13	10.7	1.76	2.33	21.8	1.33
13	Danaguri	6.38	2.24	2.85	1.01	4.58	1.85	2.48	7.85	1.72	3.67	15.4	1
14	Kalojira	6.66	2.97	2.24	1.35	4.37	2.18	2.01	7.17	1.64	2.33	21.2	2
15	Kalonunia	7.55	2.26	3.34	1.33	5.23	1.87	2.79	8.54	1.63	3	20.4	1.67
16	Khasdhan	6.47	2.24	2.89	0.96	4.64	1.88	2.46	8.36	1.8	3	19	2
17	Lilabati	7.01	2.65	2.65	1.65	5.11	2.2	2.32	8.63	1.69	2.67	16.7	1.67
18	NC 324	7.55	2.27	3.33	1.31	5.58	1.87	2.98	10.2	1.83	2.33	19.3	1.33
19	NC 365	9.2	2.42	3.81	1.81	6.34	1.95	3.25	7.4	1.17	2.33	18.3	1.67
20	Radhatilak	6.66	2.28	2.91	1.13	4.67	1.88	2.49	7.9	1.69	2.67	14.3	1.67
21	Radhunipgol	5.94	2.33	2.55	1.06	4.44	1.92	2.32	9.04	2.04	3.33	14.6	1.67
22	Radhunipgol	5.85	2.23	2.63	1.02	4.28	1.96	2.18	8.81	2.06	3.67	13.8	1.67
23	Tulaipanji	7.85	2.26	3.47	1.39	5.73	1.92	2.98	10.8	1.88	2.67	17.1	1
24	Kanakchur	6.48	2.26	2.86	1.44	4.17	2.23	1.87	6.94	1.67	3.33	15.4	1
25	Narayan purna	6.69	2.3	2.91	1.35	4.14	2.23	1.86	6.71	1.62	2.33	10.9	1.33
26	Tulsimanjari	6.57	2.17	3.02	1.26	4.65	1.87	2.49	8.13	1.75	3.33	16.6	1
27	Karnal local	11.4	2.14	5.33	1.767	8.27	1.23	4.538	13.93	1.692	7	16.15	1.67
28	Basmati 370	9.71	2.07	4.698	1.933	6.96	1.85	3.768	12.01	1.725	1	17.65	1
29	Pakistani Basmati	10.4	2.05	5.08	2.767	7.58	1.78	4.265	12.42	1.639	1	16.19	2.33
30	Pusa Basmati 1	11.2	1.88	5.965	1.867	7.91	1.59	4.978	16.85	2.129	7	22.27	3
31	Taraori Basmati	12.5	2.12	5.863	2.033	9.09	1.84	4.945	15.85	1.745	3	9.767	1.67
32	Mahisugandha	10.6	2.17	4.855	1.867	7.22	1.75	4.119	11.28	1.562	6	19.51	2
33	IR8	8.13	2.59	3.15	2.28	6.94	2.65	2.62	11.28	1.62	2	19.79	0
34	IR 36	9.51	2.38	3.99	2.28	6.79	1.8	3.75	11.57	1.78	2	22.97	0
35	IR 64	10.5	2.49	4.23	2.27	7.61	2.11	3.61	10.53	2.5	2.667	23.3	0
36	Khitish	11	2.18	2.84	2.17	8.14	1.91	2.59	11.51	1.41	2	20.13	0

Table 3. Contd.

37	Lal Swarna	7.77	2.73	2.84	1.83	5.85	2.26	2.59	8.767	1.5	2	24.6	0
38	TN 1	8.08	3.09	2.61	2.05	6.21	2.56	2.43	8.128	1.31	2	23.53	0
39	Chamarmani	9.3	2.22	4.2	1.7	6.51	1.84	3.54	11.49	1.77	2.333	18.09	0
40	Dudherswar	8.68	2.32	3.75	1.69	6.33	1.92	3.3	11.5	1.82	2.667	16.75	0
41	Satabdi	9.71	2.05	4.73	1.78	6.86	1.72	4.08	10.77	1.57	2.333	21.3	0
	Standard error	0.37	0.166	0.11	0.26	0.16	0.11	0.36	0.03	0.06	0.31	1.18	0.08

GL = Grain length; GB = Grain breadth; G-L/B = Grain length/breadth ratio; 100GW = 100 Seed. Weight; KL = Kernel length; KB = Kernel breadth; K-L/B = Kernel length/breadth ratio; KLAC = Kennel length after cooking; KER = Kernel elongation ratio; ASV = Alkali spreading value; Amy % = Amylose percentage; ARO = Aroma.

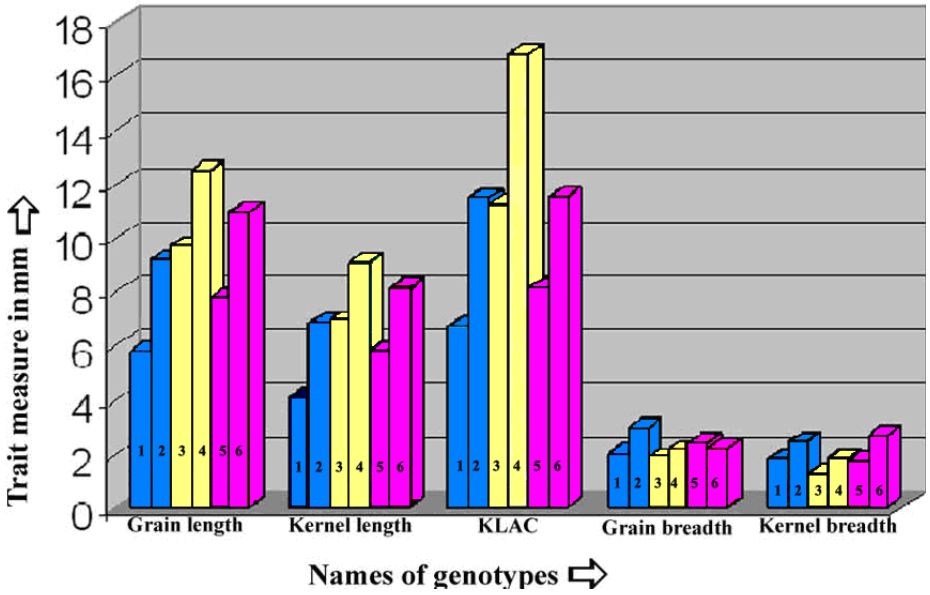

Figure 1. Minimum and maximum values of grain length, kernel length, kernel length after cooking (KLAC), grain breadth and kernel breadth among indigenous aromatic genotypes, basmati genotypes and HYV. Colour Key: Blue - Indigenous non –basmati aromatic genotypes, Yellow – Basmati genotypes, Pink – HYV. Grain length: (1) Kaminibhog (2) NC 365 (3) Basmati 370 (4) Taraori Basmati (5) Lal Swarna (6) Khitish Kernel length: (1) Chinikamini 1 (2) Narayan bhog (3) Basmati 370 (4) Taraori Basmati (5) Lal Swarna (6) KhitishKLAC: (1) Narayan purna (2) Dudherswar (3) Mahisugandha (4) Pusa Basmati 1 (5)TN 1 (6) IR 36 Grain breadth: (1) Kalogobindobhog (2) Kalojira (3) Pusa Basmati 1 (4) Mahisugandha (5) IR 36 (6)Khitish Kernel length: (1) Chamarmani (2) Kaminibhog (3) Karnal local (4) Basmati 370 (5) Satabdi (6) IR8.

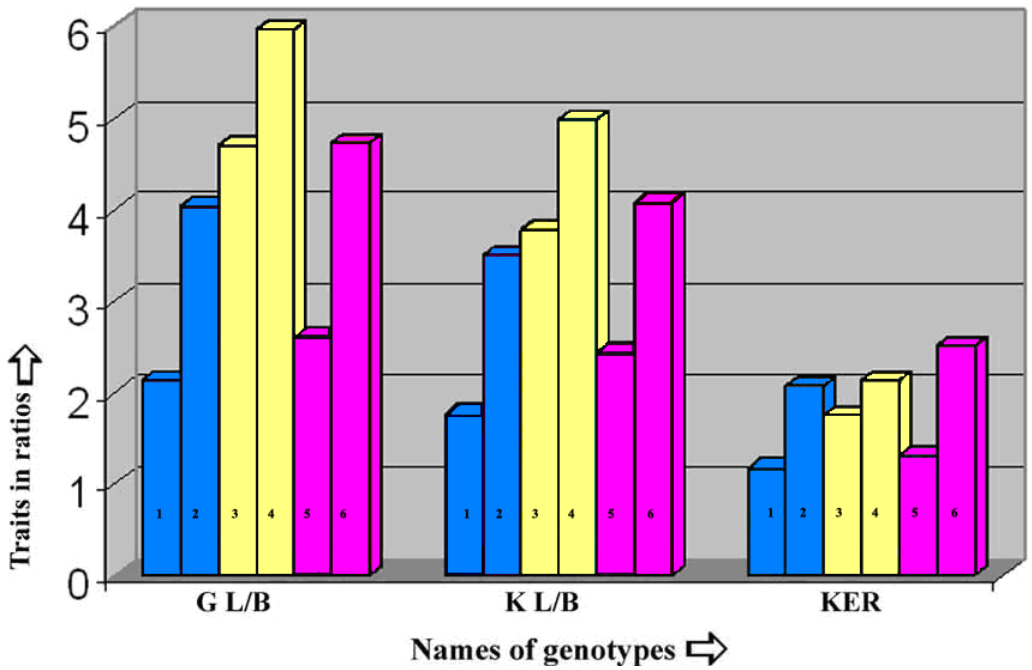

Figure 2. Minimum and maximum values of grain length/breadth (G L/ B), kernel length/breadth (K L/B) and kernel elongation ratio (KER) among indigenous aromatic genotypes, basmati genotypes and HYV. Colour Key: Blue - Indigenous non –basmati aromatic genotypes, Yellow – Basmati genotypes, Pink – HYV. Grain length/breadth (G L/ B): (1) Kaminibhog (2) Kalogobindobhog (3) Basmati 370 (4) Pusa Basmati 1 (5) TN1 (6) Satabdi. Kernel length/breadth (K L/B): (1) Kaminibhog (2) Narayan bhog (3) Basmati 370 (4) Pusa Basmati 1 (5) TN 1(6) Satabdi. Kernel elongation ratio (KER): (1) NC 365 (2) Radhunipgol (3) Taraori Basmati (4) Pusa Basmati (5) TN 1 (6) IR 64.

Table 4. Analysis of variance of the twelve traits for the 41 genotypes.

Sr. no.	Traits	Genotypes					Replication				
		df	ss	ms	F obs	F tab	df	ss	ms	F obs	F tab
1	GL	41	397.7172	9.700421	2.688548**	1	2	0.006911	0.003456	0.181998	3
2	GB	41	9.250501	0.225622	66.6854**	1	2	0.000156	7.82E-05	0.012527	3
3	GL/B	41	147.9301	3.608051	356.8048**	1	2	0.012484	0.006242	0.617303	3
4	100 GW	41	24.68526	0.602079	639.8697**	1	2	0.001094	0.000547	0.003542	3
5	KL	41	227.4919	5.548584	322.2766**	1	2	0.002221	0.00111	0.064496	3
6	KB	41	5.930815	0.144654	64.46586**	1	2	0.015646	0.007823	3.486322	3
7	KL/B	41	89.13231	2.173959	18.37229**	1	2	0.051226	0.025613	2.248472	3
8	KLAC	41	610.3587	14.8868	102.3596**	1	2	0.08141	0.040705	0.279883	3
9	KER	41	4.851455	0.118328	24.71583**	1	2	0.008395	0.004198	0.876786	3
10	ASV	41	181.8537	4.435455	28.60619**	1	2	0.308943	0.154472	0.996255	3
11	AMY %	41	1387.296	33.83648	20.13345**	1	2	1.720299	0.86015	0.511808	3
12	ARO	32	10.78205	0.431282	11.86178**	1	2	0.487179	0.24359	0.669958	3

** Observed F value is significant at the 0.01 level. *Observed F value is significant at the 0.05 level. GL = Grain length; GB = Grain breadth; G-L/B = Grain length/breadth ratio; 100GW = 100 Grain weight; KL = Kernel length; KB = Kernel breadth; K-L/B = Kernel length/breadth ratio; KLAC = Kernel length after cooking; KER = Kernel elongation ratio; ASV = Alkali spreading value; Amy % = Amylose percentage; ARO = Aroma. df = Degrees of freedom; ss = Sum of squares; ms = Mean sum of squares; F obs = Observed F value; F tab = Tabulated F value.

is almost the same as the basmati genotype and that of high yielding varieties is the highest. The result of analysis of variance of the 12 traits is given in Table 4 and it shows that all the 12 traits vary significantly between the genotypes. Table 5 shows the correlation coefficients among the 12 grain and kernel traits. Here

Table 5. Correlation coefficients among the 12 grain and kernel traits of 41 genotypes.

Traits	GL	GB	G L/B	GW	KL	KB	K L/B	KLAC	KER	ASV	AMY	ARO
GL	1	-0.53**	0.942**	0.783**	0.986**	-0.199	0.921**	0.923**	-0.249	0.34	-0.041	0.123
GB	-0.53**	1	-0.743**	-0.171	-0.542**	0.291	-0.585**	-0.57**	-0.02	-0.414*	0.259	0.007
G L/B	0.942**	-0.743**	1	0.655**	0.936**	-0.24	0.902**	0.902**	-0.171	0.481**	-0.09	0.129
GW	0.783**	-0.171	0.655**	1	0.766**	-0.225	0.743**	0.659**	-0.306	0.022	0.002	0.182
KL	0.986**	-0.542**	0.936**	0.766**	1	-0.177	0.919**	0.946**	-0.206	0.322	-0.058	0.118
KB	-0.199	0.291	-0.24	-0.225	-0.177**	1	-0.539**	-0.259	-0.389*	-0.208	-0.023	-0.356*
K L/B	0.921**	-0.585**	0.902**	0.743**	0.919**	-0.539**	1	0.918**	-0.006	0.397*	-0.019	0.28
KLAC	0.923**	-0.57**	0.902**	0.659**	0.946	-0.259*	0.918**	1	0.101	0.412*	0.007	0.22
KER	-0.249	-0.02	-0.171	-0.306	-0.206	-0.389	-0.006	0.101	1	0.219	0.147	0.336
ASV	0.34	-0.414*	0.481*	0.022	0.322	-0.208	0.397*	0.412*	0.219	1	0.14	0.307
AMY %	-0.041	0.259	-0.09	0.002	-0.058	-0.023	-0.019	0.007	0.147	0.14	1	0.135
ARO	0.123	0.007	0.129	0.182	0.118	-0.356*	0.28	0.22	0.336	0.307	0.135	1

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). GL = Grain length; GB = Grain breadth; G-L/B = Grain length/breadth ratio; 100GW = 100 Grain weight; KL = Kernel length; KB = Kernel breadth; KS = Kernel shape; K-L/B = Kernel length/breadth ratio; KLAC = Kernel length after cooking; KER = Kernel elongation ratio; ASV = Alkali spreading value; Amy % = Amylose percentage; ARO = Aroma.

we can see a highly significant positive correlation amongst grain length, kernel length and kernel length after cooking. This means they are directly proportional to each other, excepting a few cases. Grain breadth on the other hand is highly significantly negatively correlated with grain length, grain length/breadth ratio, kernel length, kernel length after cooking and amylose content.

Assessment of polymorphism from D^2 values

D^2 values were calculated from the data-set of mean values of grain and kernel traits and the dendrogram (Figure 3) obtained from the D^2 values show 2 super clusters CI and CII. Super cluster CI is divided into 2 major clusters CIA and CIB. Major cluster CIA is divided into two minor clusters 1 and 2 and minor cluster CIA1 is again subdivided into three clusters i, ii, and iii. Super clusters CI include the 26 indigenous aromatic genotypes, 3 basmati and 9 check genotypes. Super clusters CII consist of 3 basmati genotypes only, out of which two are traditional genotypes and 1 is an evolved basmati. (For abbreviations of genotype names, refer to Table 7)

Assessment of polymorphism from SSR profiles

A total of 172 alleles were identified from the 41 genotypes using 23 SSR markers. Nine null alleles and 28 rare alleles were identified during the study. Table 6 gives name of SSR marker, the maximum and minimum band length, number of alleles, number of null alleles, name of genotypes having null alleles, number of rare alleles, name of genotypes having rare alleles and PIC values for each SSR markers. 1/0 matrix was calculated from the presence or absence of polymorphic bands derived from the SSR marker profiles, and the subsequent dendrogram (Figure 4) showed two super clusters CI and CII. Super cluster CI incorporates all the nine check genotypes and is divided into two major clusters CIA and CIB. The second major cluster CII contains all the indigenous non-basmati aromatic rice and basmati rice genotypes. This cluster is also divided into two major clusters A and B. The cluster CIIA is divided into sub clusters CIIA1 and CIIA2, wherein the former includes five indigenous aromatic genotypes from West Bengal and the latter includes 5 basmati genotypes. Cluster CIIB is also divided into two sub clusters 1 and 2. CIIB1 contains two genotypes, Tulsibhog (TBHOG) an indigenous aromatic genotype from West Bengal and Taraori Basmati (TRBM), a basmati genotype. Cluster CIIB2 is again divided into two sub clusters i and ii and each of them contains 10 indigenous aromatic genotypes from West Bengal. (For abbreviations of genotype names, refer to Table 7)

DISCUSSION

As was observed from the measurement of grain and kernel traits, most of the indigenous non-basmati aromatic rice genotypes from West Bengal have strikingly smaller grain and kernel length than the basmati genotypes. The basmati genotypes on the other hand have extra long grain and kernel and they are also more slender in shape (G L/B and K L/B ratio high). The genotype NC 365 has grain length of 9.2 mm, almost the same as Basmati 370. However the most slender grain and kernel among the indigenous aromatic genotypes belong to the genotypes Kalogobindobhog and Narayanbhog respectively. The aroma levels were not

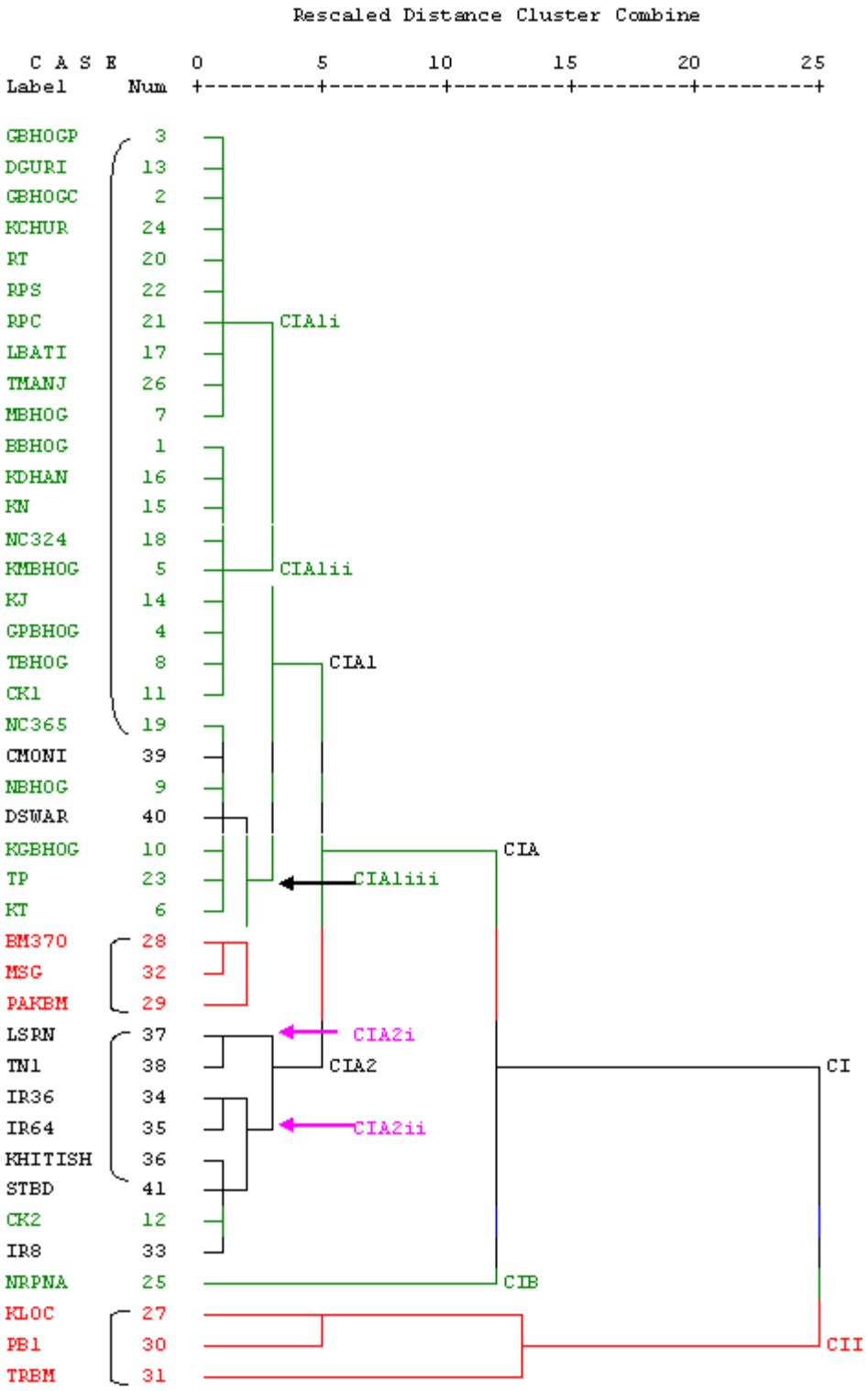

Figure 3. Dendrogram from D^2 values. Key as follows: Green – Non basmati indigenous aromatic rice; Red – Basmati rice; Black – check genotypes

markedly different between the indigenous non-basmati aromatic rice and the basmati group. Among the basmati genotypes Pusa Basmati 1 has the highest level of aroma and among the indigenous non-basmati aromatic genotypes; Gobindobhog has the same level of aroma. Pusa Basmati 1 also has the most slender grain and

Table 6. Name of SSR marker, maximum and minimum band length, number of alleles, number of null alleles, name of genotypes having null alleles, number of rare alleles, name of genotypes having rare alleles and PIC values

Sr no.	SSR marker	Mol wt max (bp)	Mol wt min (bp)	A	N	Names of genotypes having the null alleles	R	Names of genotypes having the rare alleles	PIC value
1	RM 42	172.984	142.095	5	0		1	Basmati 370	0.728138
2	RM 44	160.127	100.6	5	3	Lilabati, Basmati 370, Karnal local	1	Tulsimanjari	0.706371
3	RM 72	229.244	132.524	8	0		2	Kaminibhog, Narayanpurna	0.804283
4	RM 80	155.195	110.51	10	2	Badshahbhog, Lilabati	2	Khasdhan, Taraori Basmati	0.866535
5	RM 112	171.199	118.109	4	0		0		0.71624
6	RM149	287.08	237.321	10	0		2	Chinikamini 2, Kalojeera	0.816181
7	RM 152	168.407	105.047	8	0		2	Tulsibhog, Taraori Basmati	0.803688
8	RM 182	321.414	293.386	12	0		2	Tulsibhog, Tulsimanjari	0.902439
9	RM 207	170.27	75.817	8	0		2	Tulaipanji, Kalogobindobhog	0.813206
10	RM 210	174.11	134.675	6	0		1	Chamarmoni	0.757882
11	RM 218	153.252	118.643	5	0		0		0.704938
12	RM 223	174.462	119.846	12	0		2	Tulsibhog, Chamormoni	0.909578
13	RM 250	158.44	115.218	5	1	Mahisugandha	0		0.73125
14	RM 251	147.165	110.741	9	1	Lilabati	1	Kaminibhog	0.808125
15	RM 282	140.385	127.356	3	0		0		0.598453
16	RM 284	151.4	114.107	6	0		1	Taraori basmati	0.760262
17	RM 310	207.085	96.232	9	1	Pakistani basmati	1	Chamarmoni	0.883125
18	RM 337	377.771	154.915	10	0		2	Danaguri, NC324	0.839381
19	RM 339	190.187	126.915	5	0		0		0.777513
20	RM 341	227.015	87.068	10	0		2	Tulsibhog, Taraori Basmati	0.892326
21	RM 505	197.657	116.192	9	0		2	Tulaipanji, Tulsimanjari	0.804283
22	RM 530	184.163	140.184	5	0		1		0.671624
23	RM 569	196.329	136.261	8	1	Lilabati	1		0.8575
Total	-	-		172	9		28		-

A- Number of alleles, N - null alleles, R – rare alleles, PIC – polymorphism information content.

kernel and the kernels elongate the most after cooking. It is the combination of extra long grains and kernels and high aroma, present in the basmati genotypes; which is highly priced in the national and international market and making them more costly than the indigenous non-basmati aromatic rice genotypes from West Bengal.

The alkali spreading value (ASV) of the basmati genotypes range from 1 to 7 and that of the indigenous aromatic genotypes range between 2.33 to 3.33. Since ASV is inversely proportional to the gelling temperature of rice starch, it is also a measure of cooking time (Little et al., 1958). Hence it follows that basmati genotypes with ASV 6 or 7, like Mahisugandha and Pusa Basmati 1, cook faster than the other genotypes of this study. This feature makes Pusa Basmati 1 specially suited for certain rice delicacies and also for cooking in a lesser amount of water. The indigenous aromatic genotypes have high gelling temperature and a longer cooking time. The trait amylose content (measured in percentage) is directly proportional to stickiness of cooked rice kernels (Jennings et al., 1979). In general the basmati genotypes are less sticky than the indigenous non-basmati aromatic rice genotypes.

Table 7. Abbreviation of genotype names used in Figures 1 and 2.

Sr. no.	Genotype name	Abbreviation	Sr. no.	Genotype name	Abbreviation
1	Badshahbhog	BBHOG	22	Radhunipgol 1	RP1
2	Chinikamini 1	CK1	23	Radhunipgol 2	RP2
3	Chinikamini 2	CK2	24	Tulaipanji	TP
4	Danaguri	DGURI	25	Tulsibhog	TBHOG
5	Gobindobhog 1	GBHOG1	26	Tulsimanjari	TMANJ
6	Gobindobhog 2	GBHOG2	27	Basmati 370	BM370
7	Gopalbhog	GPBHOG	28	Karnal local	KLOC
8	Kalogobindobhog	KGBHOG	29	Mahisugandha	MSG
9	Kalojira	KJ	30	Pakistani Basmati	PAKBM
10	Kalonunia	KN	31	Pusa Basmati 1	PB1
11	Kanakchur	KCHUR	32	Taraori Basmati	TRBM
12	Kaminibhog	KMBHOG	33	IR-8	IR8
13	Kataribhog	KT	34	IR-36	IR36
14	Khasdhan	KDHAN	35	IR-64	IR64
15	Lilabati	LBATI	36	Khitish	KTS
16	Mohanbhog	MBHOG	37	Satabdi	STBD

Some of them like Kaminibhog and Kalonunia have higher amylose percentage and are stickier than Gobindobhog and Radhunipagol. The more sticky genotypes are used mainly for making desserts, while the others are used as prime table rice.

From the ANOVA tables (Table 4), it is evident that the means of the measured traits vary significantly among the 41 rice genotypes included in this study. Hence, as far as the twelve traits in this study are concerned, each genotype is distinct from the other.

The 23 SSR markers used for this study are previously mapped (Temnykh et al., 2000). Most of the markers were selected from chromosome 8 as two important traits of aromatic and basmati rice, aroma (Ahn et al., 1992) and cooked kernel elongation ratio (Ahn et al., 1993), had been mapped earlier using RFLP markers to chromosome eight. All the SSR markers used for this study revealed a clear and consistent amplification profile. The results are consistent with published reports on microsatellite frequency in the rice genome (Blair et al., 1999; Temnykh et al., 2000; McCouch et al., 2002). Stutter bands, which are minor products amplified in PCR that have lower intensity than the main allele and normally lacks or has extra repeat units were also present in the profiles of most of the markers used. Null alleles were present probably due to mutations in the binding region of one or both of the microsatellite primers, thereby inhibiting primer annealing (Callen et al., 1993).

In Figure 3, the indigenous non-basmati aromatic genotypes, the HYVs and three basmati genotypes have been grouped into one super cluster. The other three basmati genotypes namely, Karnal local, Pusa basmati 1 and Taraori basmati have been grouped separately into a second super cluster. Out of these three, Pusa basmati 1 is an evolved basmati and also has the highest values for 6 traits. The dendrogram from the SSR profiles (Figure 4) have clearly demarcated the group of indigenous non-basmati aromatic rice and the basmati genotypes from the group of HYVs check varieties. The check varieties are all non aromatic and the values of their grain and kernel dimensions are an average between the short indigenous aromatic and extra long basmati genotypes. Among the aromatic rice five of the basmati rice genotypes have formed a separate cluster indicating their genetic distinction from the rest of the aromatic members. The genotype collection had two entries each with the name Radhunipagol and Gobindobhog collected from separate sources as given in Table 1. Both entries for Radhunipagol are clustered together suggesting that they have very little genetic difference between them. In case of Gobindobhog, the entries are widely diverged. Such differences are possibly due to environmental variations and have been previously documented by other workers (Glaszmann, 1987; Katiyar and Singh, 1990).

The basmati genotypes included in this study were genetically distinct as far as, the used 23 pairs of marker loci are concerned. According to international market demand, they were also superior on account of the twelve grain and kernel traits. Pusa Basmati 1 outperforms most of the indigenous non-basmati aromatic rice genotypes from West Bengal in aspects of kernel length/breadth, kernel length after cooking, kernel elongation ratio, alkali spreading value and aroma. The indigenous aromatic genotypes are however almost at par with the basmati genotypes as far as the levels of aroma are concerned. Also, it is a traditional observation that the indigenous aromatic genotypes are more versatile from the culinary perspective and has a variety of end use - as table rice, desserts, as offerings to God in various ceremonies, as diet for the convalescent and for

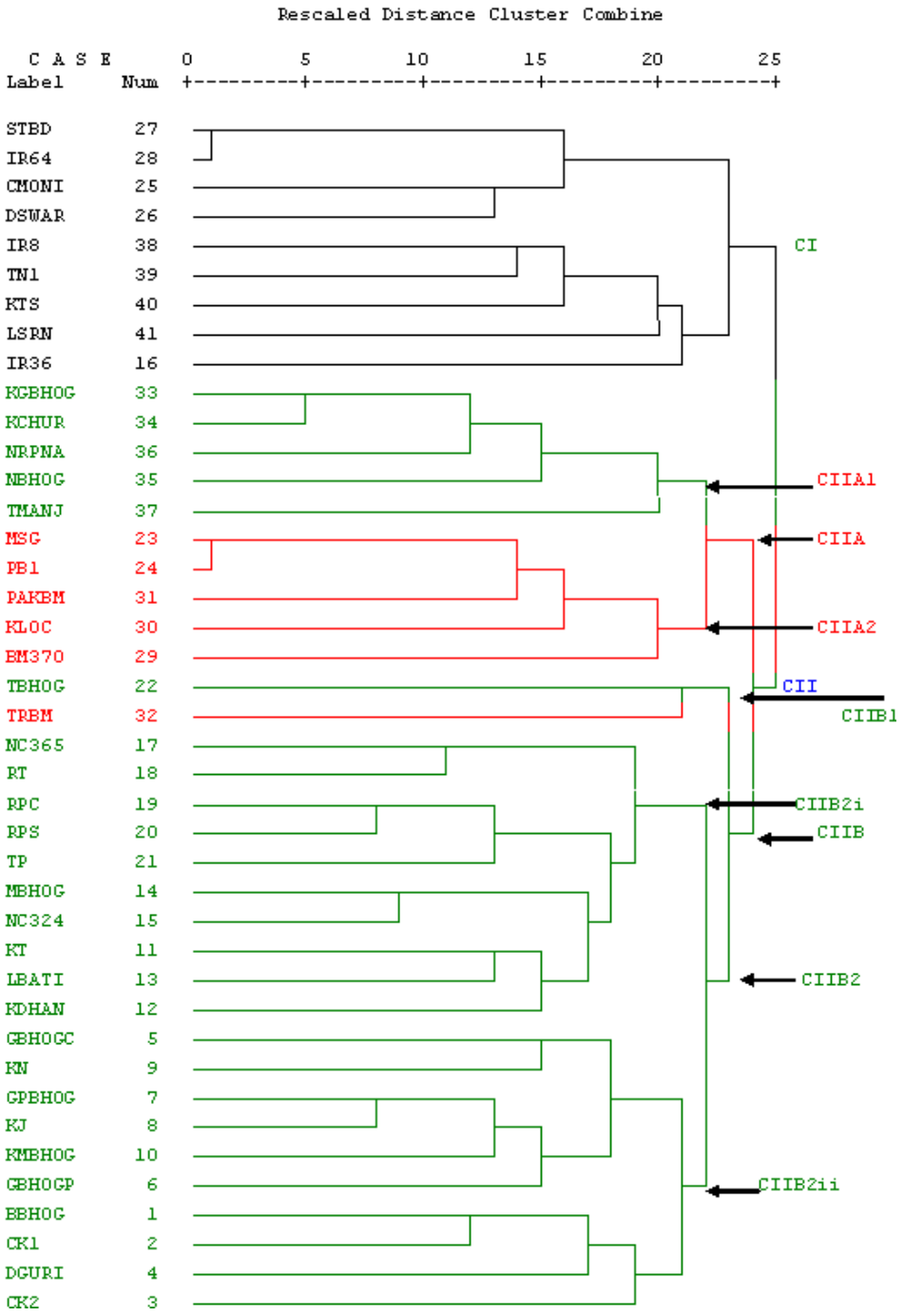

Figure 4. Dendrogram from SSR profiles. Key as follows: Green – Non basmati indigenous aromatic rice; Red – Basmati rice; Black – check genotypes.

making popped rice. They are well adapted to the agro climatic conditions of their respective place of cultivation and are a distinctive part of the culture and socio economic structure of the agrarian population (Rekha et al., 2011). Improvement of commercial status and preservation of biodiversity present among such indigenous non-basmati aromatic rice is also linked to the conservation of the long heritage associated with it.

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