Molecular Characterization of Genetic Diversity in Glutinous Rice of Assam using RAPD Marker

RN Sarma* and D Barooah
Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat-785013

The genetic diversity of 60 glutinous rice accessions of Assam was analyzed using 17 RAPD primers. Out of the 160 reproducible bands, 145 found to be polymorphic. The number of polymorphic bands ranged from 4 (OPL-17) to 18 (OPB-07). With an average of 88.75% polymorphism between the accessions, a high degree of molecular variation was detected in the rice collection. The average Jaccard's similarity co-efficient based on RAPD data was found to be 0.446, indicating a high level of diversity in the glutinous rice of Assam. The cluster analysis grouped the accessions in to nine sub clusters. Implications of these findings in indigenous glutinous rice conservation and improvement are discussed.

Key words: Glutinous rice, Bora rice, Chakuwa rice, Genetic diversity, Assam rice, RAPD

Among different classes of rice available in Assam, glutinous or waxy rice (locally known as 'Bora' rice) is an important class. This class, with higher amylopectin than amylose, is considered as soft rice because of its soft cooking consistency. Based on amylose content, the glutinous rice of Assam has been classified into two groups, Bora and Chokuwa. The amylose content of Chokuwa varieties varies between 15 and 20%, while that in Bora varieties found to be in trace (Dutta and Barua, 1978). Glutinous rice or Bora rice of Assam has significance in social and religious ceremonies and forms a popular daily breakfast diet in rural Assam. The local people use Bora rice in the preparation of snacks, flat rice, puffed rice, sweet rice beer and other dishes. The Bora rice of Assam has the potential of greatly increasing exports into this market, owing to its cooked stickiness (i.e. low amylose content), which is preferred by the South East Asian market.

Bora rice germplasm of Assam constitute an important source of genetic variation for breeding of improved Bora variety/hybrid, and their conservation and characterization is an important component in this direction. Though some of the germplasm of this important class of rice has been maintained in Assam Agricultural University (AAU) and elsewhere, no effort has been made to study the nature and extent of diversity along with possibility of redundant accessions in *ex-situ* conserved materials (Barooah and Sarma, 2004). The difficulty of studying diversity based on morphological data is well demonstrated in various studies (Ahmed and Das, 1990; Das and Ahmed, 1992). Barooah and Sarma (2004) reported the use of molecular marker in diversity analysis of Assam rice, particularly in sali rice (winter rice). The use of molecular marker data alone or in combination with morphological characterization is considered as best to characterize the genetic diversity.

There is very little information available on the nature and extent of genetic diversity of Bora rice of Assam, particularly at the molecular level. Hence the present study was conducted to characterize and document the genetic variation by identifying the DNA profile in few glutinous rice accessions of Assam using RAPD marker.

Material and Methods

Sixty glutinous rice accessions, including Bora and chokuwa types, were obtained from Regional Agricultural Research Station, Assam Agricultural University, Titabar. The list of the accessions with their place of collection is given in Table 1. Some of the accessions with same name have been maintained because of inability to distinguish them on the basis of morphology.

Genomic DNA was isolated from seeds following Plaschke et al (1995) with minor modification. Two to three seeds of each accession were crushed with a mortar and pestle to a fine powder for DNA extraction using 1000 ul of extraction buffer (1M Tris-Cl, pH 8.0, 5M NaCl; 500mM Na_2 EDTA; 20%SDS). The homogenate was centrifuged to remove cell debris. The supernatant was treated with RNase and DNA was precipitated with chilled 95% alcohol. The quality and quantity was assayed by running DNA on a 0.8% agarose gel alongside a known quantity of lambda uncut DNA.

*Author for correspondence: E-mail: masarma@aau.ac.in,ramen5in@yahoo.co.in

Table 1. Glutinous rice accessions used in the study

Sl. No.	Name	Place of collection	Sl. No.	Name	Place of collection
1.	Aghoni Bora	High yielding variety	31	Kmj B 2	Karimganj
2	Bao Bora	Dhakuakhana, Lakhimpur	32	Kmj B 48	Karimganj
3	Beji Bora	Dhakuakhana, Lakhimpur	33	Kmj B 9	Karimganj
4	Boga Bora 1	Rowraih, Jorhat	34	Kola Bora	Titabar, Jorhat
5	Boga Bora 2	Nagaon	35	Malbhog Bora	Jorhat
6	Bogachokua	Titabar, Jorhat	36	Memon Bora	Lakhimpur, Lakhimpur
7	Bor Bora	Golaghat, Golaghat	37	Moju Chokua	Titabar, Jorhat
8	Bor Chokua	Jorhat, Jorhat	38	Mon Bora 1	Saraibahi, Jorhat
9	Bora 1	Nalbari, Kamrup	39	Mon Bora 2	Borhola, Titabar
10	Bora 2	Silghat, Nagaon	40	Nae lace Bora	Golaghat, Golaghat
11	Bora 3	Jagiroad, Morigaon	41	Nalia Bora	Kampur, Nagaon
12	Bora 4	Dibrugarh	42	New Bora 2	Nagaon
13	Bora 5	Sibsagar	43	New Bora 5	Nagaon
14	Bormalbhog	Goalpara	44	New Bora 6	Kampur, Nagaon
15	Chandra Bora	Jorhat, Jorhat	45	New Bora 7	Lakhimpur, Lakhimpur
16	Chokua Bora	Titabar, Jorhat	46	New Bora 8	Nagaon
17	Ghew Bora	Batadrava, Nagaon	47	Poita Bora	Rangajan, Golaghat
18	Goruckokua Bora	Janji, Jorhat	48	Ranga Bora	Titabar, Jorhat
19	Helochi Bora	Dergaon, Jorhat	49	Rangali Bora	Titabar, Jorhat
20	Jangoni Bora	Jorhat	50	Runohi Bora	Bokakhat, Golaghat
21	Joha Bora	Sibsagar	51	Rupahi Bora	Batadrava, Nagaon
22	Jota Bora	Golaghat	52	Sam Chokua	Dergaon, Jorhat
23	Kajoli Bora 1	Rupahi, Nagaon	53	Saudung Bora	Borhola, Titabar
24	Kajoli Bora 2	Sibsagar	54	Sukoni Bora 1	Borhola, Jorhat
25	Kakhichakua Bora	Nagaon	55	Sukoni Bora 2	Titabar, Jorhat
26	Kauri Bora	Titabar, Jorhat	56	Tagun Bora	Kochajan, Titabar
27	Khaldhor Bora	Jorhat	57	Til Bora 1	Golaghat
28	Khamti Bora	Arunachal Pradesh	58	Til Bora 2	Dergaon, Jorhat
29	Kmj B 10	Karimganj	59	Titanhulia Bora	Dhemaji, Lakhimpur
30	Kmj B 13	Karimganj	60	Tulasi Bora	Rupahi, Nagaon

Amplification reaction was carried out in a 25ul reaction volume containing 10ng of template DNA, 50pM of primer, 200μM of dNTPs, 0.5 unit of Taq polymerase (Bangalore Genei Pvt. Ltd, Bangalore, India) and 1XPCR Buffer (50mM KCl, 10mMTris-Cl, 2mM, 0.01% gelatin). Initially thirty decamer random primers (Operon Technologies, Inc., Alamanda, CA, USA) were used for RAPD amplification as described by Williams *et al.* (1990) with minor modification. Amplification reaction was performed in a thermal cycler (Gene amp PCR2400, Applied Biosystem, USA) for 35 cycles. Each cycle consisted of denaturation for 1 minute at 94°C, 1 min annealing at 37°C, followed by a 5 min for primer elongation at 72°C, and final elongation for 1 min at 72°C. The PCR products were resolved by electrophoresis in 1.5% agarose gel in 1XTBE buffer, ethidium bromide stained gel was photographed with a digital gel documentation system (Ultra-violet products, UK). Reproducibility of RAPD assay was tested by performing duplicate reactions at different times using identical genotypes and primer combinations under strict control of experimental conditions and only the reproducible bands were scored.

The RAPD bands were scored as present (1) or absent (0) for each genotype-primer combination of all the 60 glutinous rice accessions, considering each amplified band as unique locus. Band-sharing data were used to calculate genetic similarities using Jaccard's coefficient (Jaccard, 1908). The similarity matrices were analyzed using NTSYS-PC 2.10 (Rohlf, 2002) and clustered with UPGMA (un-weighted pair group methods using arithmetic average) algorithm to determine the genetic relationships of the 60 Bora rice accessions and the dendrogram was constructed using NTSYS-PC. The average similarity index for all pair wise comparison ($\bar{X}_D$) was calculated and used to estimate the probability of DNA fingerprints of two genotypes being identical by chance (Wetton et al. 1987, Ramkishna et al. 1994) with the formula $(\bar{X}_D)^n$, where $(\bar{X}_D)$ = average similarity index and n= the average number of bands per accession.

Results and Discussion

Out of the 30 primers tested, 17 primers were selected to detect polymorphism in glutinous rice accessions based on their reliability of amplification profile. The amplification profile, generated by the 17 primers, is summarized in Table 2. The number of polymorphic

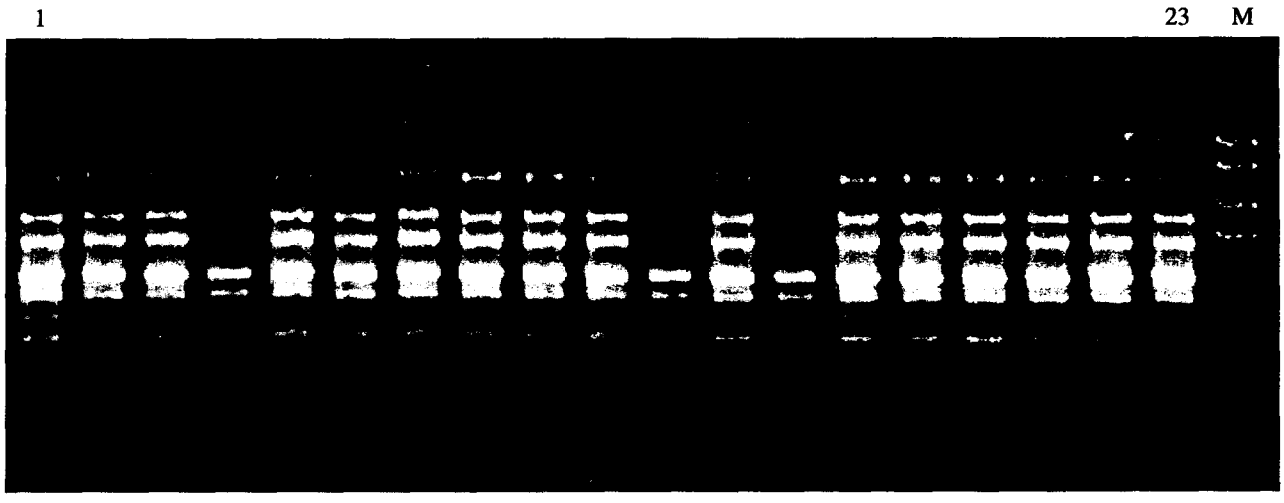

Fig.1. A representative RAPD amplification profile in glutinous rice of Assam with primer OPB08. Numbers (from 1to 23) represent the accessions as per Table 1.

Table 2. Summary of RAPD data of 60 glutinous rice accessions

Primers	Total band	Polymorphic bands	Polymorphism (%)
OPB 07	18	15	83.33
OPB 08	9	8	88.89
OPB 10	11	10	90.91
OPD 01	7	7	100.00
OPD 03	8	8	100.00
OPD 18	9	8	88.89
OPD 20	13	11	84.62
OPH 04	7	7	100.00
OPH 05	9	8	88.89
OPH 07	13	12	92.31
OPH 19	9	8	88.89
OPH 20	9	9	100.00
OPK 14	6	2	33.33
OPK 19	8	8	100.00
OPL 17	4	3	75.00
OPM 16	10	9	90.00
OPP 02	10	9	90.00
Total	160	142	88.75

bands ranged from 4 (OPL-17) to 18 (OPB-07) with an average of 88.75% polymorphism. The size of amplified products ranged from 0.05 kb to 2.5 kb.

The average similarity index, based on Jaccard co-efficient, was 0.446, indicating that existing diversity was sufficient to distinguish any given two accessions. The similarity co-efficient ranged from 0.275 (between 'Titanhulia Bora' and 'Bor Bora') to 0.840 (between 'New Bora 8' and 'Bora 2'). The place of collection revealed that the two most dissimilar accessions were collected from two different districts on either side of Brahamputra valley, whereas, the two most similar accessions came from same district. The phenetic representation of similarity co-efficient among 60 glutinous rice accessions is presented in Figure 2. The dendogram classified 60 glutinous rice genotypes into two major clusters (A and B) with 42 and 18 accessions, respectively. The major group A could be classified into seven sub clusters. The sub cluster A1 comprised of seven genotypes, in which 'Bora 2' and 'New Bora 8' (from Nagaon district with 0.84% similarity) was grouped together, indicating close genetic similarity between these accessions. The sub cluster A2 comprised of 11 accessions, out of which six were semi-glutinous or chokuwa accessions ('Sam Chokua', 'Bor Chokua', 'Chokua Bora', 'Goruckokua Bora', 'Moju Chokua' and 'Bogachokua'). The clustering pattern in this sub cluster revealed that semi-glutinous accessions showed some difference from glutinous accession. Despite most of accessions were collected from same district, the average similarity of 0.465 among the chokuwa accessions indicated sufficient genetic differences among themselves. The sub-clusters A3 and A4 consisted of nine genotypes each. In thse two sub-clusters, the accessions from Borak valley of Assam were included. The three sub clusters, A5, A6 and A7, were comprised of two genotypes each.

The cluster B comprised of two sub-clusters, and the 'Bora 3' showed sufficient genetic divergence from the other genotypes in this cluster. Seven accessions form another sub cluster B1, in which 'Nalia Bora' (collected from Nagaon) and 'Kauri Bora' (collected from Golaghat) were grouped together with 0.834% similarity revealing some genetic similarity between them. In this sub cluster 'Aghoni Bora' and 'Bora 1' showed divergence from the rest. It is worthwhile to

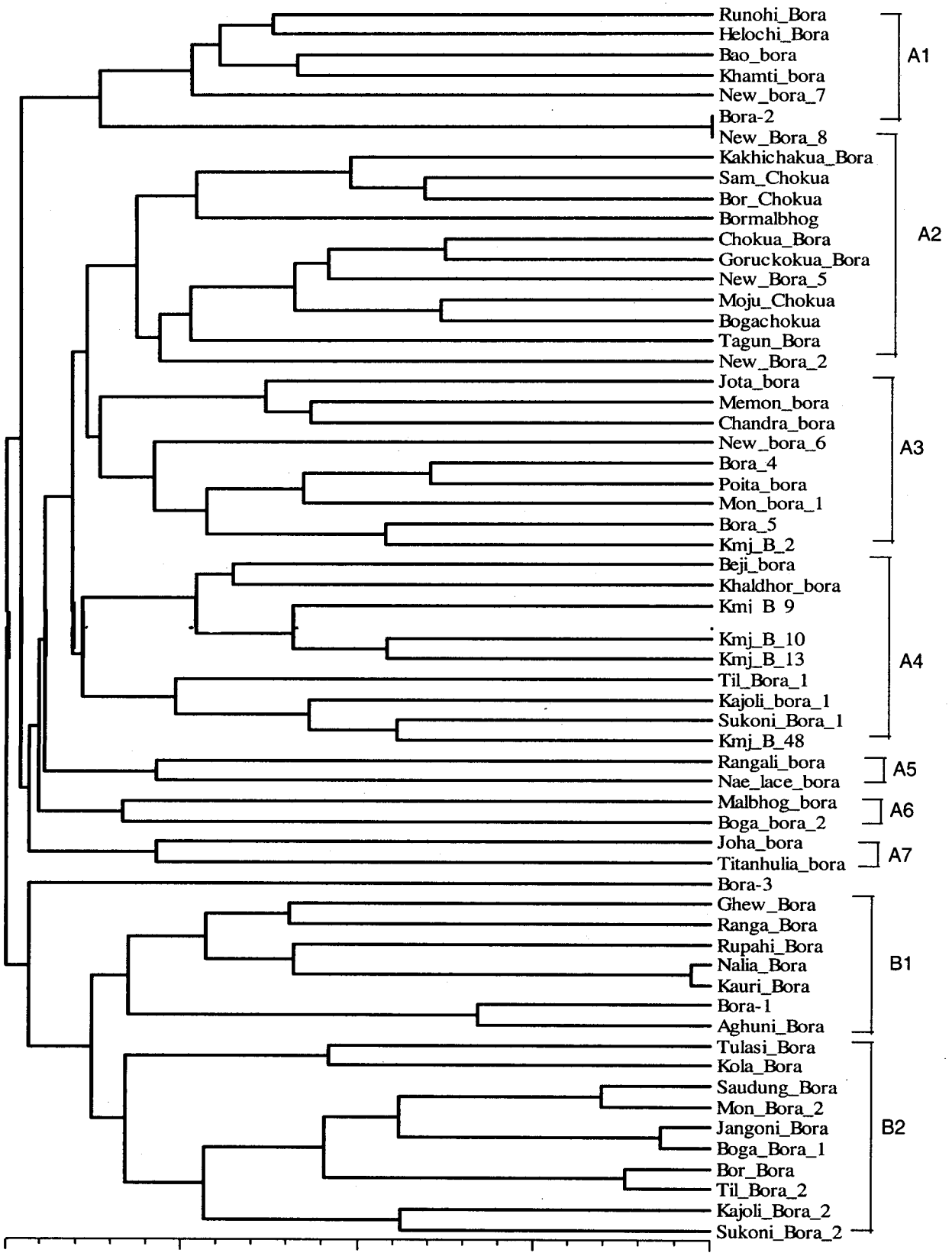

Fig.2: Dendrogram of 60 glutinous rice genotypes constructed using UPGMA based on 160 RAPD bands using Jaccard's coefficient of similarity

mention that 'Aghoni Bora' is a high yielding variety developed in AAU (pedigree 'Gandhi Bora' x 'Kmj-1-52-2') (Pathak, 2001). The sub cluster B2 includes ten accessions, in which 'Kajoli Bora 2' and 'Sukoni Bora 2' showed genetic divergence from the rest of accessions. Some of accessions with common names (e.g. 'Kajoli Bora 1' and 'kajoli Bora 2' with 43% similarity); ('Boga Bora 1' and 'Boga Bora 2' with 44% similarity) were found to be different, based on RAPD data.

This study revealed existence of sufficient genetic variation at DNA level in Bora rice collection maintained in Assam Agricultural University. This clustering pattern was not associated with geographic locations. Genetic drift and unconscious selection from original land races might have contributed to this situation. The probability of banding pattern being identical by chance in any two Bora accession was 4.39×10^{11}, suggesting that about 10^{11} Bora accession can be distinguished with the 17 primers used. This indicates the high resolving power of RAPD markers to distinguish Bora rice accession of Assam. Knowledge about the distribution of genetic variation among the Bora landraces plays crucial role in their conservation and utilization in breeding programme. However, accessions that are found similar based on molecular genetic data may differ in just one important trait. So before declaring similar accessions as redundant, evaluation data and end user request should be considered or otherwise molecular data should be considered to identify those as redundant (Treuren *et al.*, 2001).

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