Bioremediation, Biodiversity and Bioavailability ©2010 Global Science Books

Assessment of Genetic Diversity in Banana (*Musa* spp.) of North-Eastern India by RAPD

Saha Roy Olivia[1] • Bantawa Pranay[1] • Ghosh Swapan Kumar[1] • Tripathi Shashi Bhushan[2] • Ghosh Partha Deb[3] • Mondal Tapan Kumar[1*]

[1] Biotechnology Laboratory, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, 736165, India
[2] Plant Molecular Biology Lab, Tata Energy Research Institute, India Habitat Centre, Lodi Road, Delhi-110003, India
[3] Department of Botany, University of Kalyani, Kalyani, Nadia, West Bengal, India

Corresponding author: *mondaltk@yahoo.com

ABSTRACT

Genetic diversity was studied among 28 landraces of banana collected from traditional farming areas of North Bengal and North East India using Random Amplified Polymorphic DNA (RAPD) markers. Evaluation of genetic diversity is essential for conservation and management and to trace hybrids as well as duplicate entries. The PCR products produced by 10 polymorphic primers revealed 66 bands, 55 of which were polymorphic (72.7%). A dendogram was constructed based on Dice's coefficient matrix by unweighted pair-group mean analysis (UPGMA) using NTSYS-PC, which reveal two major groups of banana: plains (North Bengal) and high hills (Arunachal Pradesh and Sikkim). The extent of variability was 86.96% and the average inter- and intra-population variability ranged from 45 to 52%. A resemblance matrix was developed using SMC, which was used with NTSYS to compute cluster analysis. To the best of our knowledge, this is the first attempt to assess genetic diversity of banana from North East India.

Keywords: genetic variation, germplasm collection, multivariate analysis, molecular marker
Abbreviations: NTSYS, numerical taxonomy system; **PCR**, polymerase chain reaction; **RAPD**, random amplified polymorphic DNA; **UPGMA**, unweighted pair group method with arithmetic mean

INTRODUCTION

The genus *Musa* is a member of the family Musaceae. The number of species within *Musa* lies between 30 and 40 (Simmonds 1995). Cheesman (1947) proposed a classification of the genus *Musa* into four sections: *Eumusa*, *Rhodochlamys*, *Australimusa* and *Callimusa*. Cheesman's classification of *Musa* provided general knowledge of the taxonomy and was used for nearly 50 years without any significant changes (De Langhe 2000). Later various changes and some major and minor regroupings of the classification of *Musa* were suggested by Wong *et al*. (2002).

Edible banana originated in South-East Asia, including India (Uma *et al.* 2005). It was simultaneously introduced into other continents also (Simmonds 1995). However, plants are mainly distributed on the margins of tropical rainforests (Wong *et al.* 2002). The green mountains of the Himalayas in North East India lie between 23° 3′ N and 28° N, 88° 3′ E and 95° E. This region is highly species-rich and is considered one of the 25 biodiversity hotspots of the world (Tandon *et al*. 2009). Banana and plantains (*Musa* spp.) originated from intra- or interspecific crosses of two wild diploid species, *Musa acuminata* and *Musa balbisiana*. These two species of *Musa* contribute the A and B genome, respectively (Simmonds 1995). Evolution in *Musa* further occurred through polyploidization and the accumulation of somatic mutations (Stover and Simmonds 1987). Therefore, most commercial bananas can present different genomic combinations such as AA, AB, AAA, AAB, ABB, AAAA, AAAB, AABB, and ABBB. It is well known that bananas and plantains have a basic chromosome number of x = 11, with 22 (diploid), 33 (triploid), 44 (tetraploid) chromosomes (Stover and Simmonds 1987). Further, in India there is a wide range of B-rich genomes (AB, AAB, ABB, and ABBB) and a great diversity of the B genome (BB) (Uma *et al.* 2005).

Because of the limitations of morphological and biochemical markers, efforts are being directed to use molecular markers for characterizing germplasm diversity. Molecular markers have demonstrated a potential to detect genetic diversity and to aid in the management of plant genetic resources (Virak *et al*. 2000; Song *et al*. 2003). In contrast to morphological traits, molecular markers can reveal differences among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management. Several types of molecular markers are available today to characterize *Musa* groups, including those based on restriction fragment length polymorphism (RFLP) (Jarret *et al.* 1992; Bhat *et al.* 1995), random amplified polymorphic DNA (RAPD) (Howell *et al.* 1994; Bhat *et al*. 1995; Ongusa *et al.* 2004; Agoreyo *et al.* 2008), amplified fragment length polymorphism (AFLP) (Loh *et al.* 2000; Ude *et al.* 2002), simple sequence repeats (SSRs) (Ge *et al.* 2005) and variable number tandem repeats (VNTRs) (Kaemmer *et al.* 1993). However, the RAPD method of DNA fingerprinting is widely used in conservation biology because of quick results, cost-effectiveness and reproducibility (Williams *et al.* 1993). Morphological, biochemical and RAPD analyses have been carried out by several researchers for the evaluation of genetic diversity in banana (Bhat and Jarret 1995; Pillay *et al.* 2001).

Therefore the objective in our present study was to develop genetic fingerprints of *Musa* cultivars of North Bengal as well as the North-Eastern part of India. To the best of our knowledge, no molecular characterization has been conducted to assess the genetic variation of *Musa* spp. in these areas.

Received: 3 September, 2009. Accepted: 26 November, 2009.

Original Research Paper

Table 1 Banana landraces used in the present study.

Accession №	Local name	Altitude m	Place of collection	Mean latitude	Mean longitude
B1	Sikkim local 1	1547	Sikkim	27º20′ N	88º 37′ E
B2	Sikkim local 2	1547	Sikkim	27º20′ N	88º 37′ E
B3	Sikkim Local 3	1547	Sikkim	27º20′ N	88º 37′ E
B4	Sikkim local 4	1547	Sikkim	27º20′ N	88º 37′ E
B5	Mungray	1547	Sikkim	27º20′ N	88º 37′ E
B6	Sikkim local 5	1547	Sikkim	27º20′ N	88º 37′ E
B7	Ghusharay	1547	Sikkim	27º20′ N	88º 37′ E
B8	Bucharay	35	North Bengal	26º22′ N	89º 29′ E
B9	Chinichamp 1	35	North Bengal	26º22′ N	89º 29′ E
B10	Jhaichamp	1547	Sikkim	27º20′ N	88º 37′ E
B11	Kaburay	1547	Sikkim	27º20′ N	88º 37′ E
B12	Darjeeling local 1	2134	North Bengal	27º03′ N	88º 03′ E
B13	Darjeeling local 2	2134	North Bengal	27º03′ N	88º 03′ E
B14	Gros Michel	750	Arunachal Pradesh	28º00′ N	95º 00′ E
B15	Hathidat	850	Arunachal Pradesh	28º00′ N	95º 00′ E
B16	Arunachal local	956	Arunachal Pradesh	28º00′ N	95º 00′ E
B17	Thorchara	35	North Bengal	26º22′ N	89º 29′ E
B18	Cooch Behar local 1	35	North Bengal	26º22′ N	89º 29′ E
B19	Cooch Behar local 2	35	North Bengal	26º22′ N	89º 29′ E
B20	Bichi Kala	35	North Bengal	26º22′ N	89º 29′ E
B21	Bichi kala type 2	35	North Bengal	26º22′ N	89º 29′ E
B22	Cooch Behar local 3	35	North Bengal	26º22′ N	89º 29′ E
B23	Kachkala	35	North Bengal	26º22′ N	89º 29′ E
B24	Manua	35	North Bengal	26º22′ N	89º 29′ E
B25	Assami manua	55	North Bengal	26º22′ N	89º 29′ E
B26	Chang manua	35	North Bengal	26º22′ N	89º 29′ E
B27	Malbhog	35	North Bengal	26º22′ N	89º 29′ E
B28	Chini champa 2	35	North Bengal	26º22′ N	89º 29′ E

MATERIALS AND METHODS

Plant materials

Detailed information of plant materials and their place of collection are listed in **Table 1**. An extensive field survey was carried out in the traditional farming villages of North Bengal and North-East India (Sikkim, Arunachal Pradesh). North Bengal lies in the Northern half of West Bengal and encircled by international boundaries of Nepal, Bhutan and Bangladesh. It offers a unique combination of high mountainous region in the north to the vast Gangetic plains in the extreme South. Sikkim is placed between 27° 5′ N to 20° 9′ N (latitude) and 87° 59′ E to 88° 56′ E (longitude) whereas Arunachal Pradesh (26° 30′ N to 29° 30′ N latitude; 91° 30′ E to 97° 30′ E longitude) is the extreme North-East state of India, which is situated in the foothills of the Himalayas. Based on the morphological study 28 *ex situ* accessions were collected from these regions. These accessions numbers are represented by using the symbols B1 to B28 in **Table 1**. Corresponding local names are also documented in column 2. Germplasm in the form of plants were maintained in the Experimental Farm at Uttar Banga Krishi Viswavidalaya, West Bengal, India. The young leaves were collected from tagged plants in a polythene bag and stored at -80°C in the laboratory, till further processing for DNA extraction.

DNA isolation and primer screening

The DNA was isolated by following the cetyl tri-methylammonium bromide (CTAB) method as explained here. Around 100 mg of tissue was powdered in liquid nitrogen with a pre-chilled mortar and pestle. The powder was immediately transferred to an Eppendorf tube (1.5 ml) containing 700 µl of fresh extraction buffer (100 mM Tris Hcl, pH 8.0; 20 mM EDTA pH 8.0; 1.4 M NaCl), 2% CTAB (w/v), 2 µl of β-marcaptoethanol, and 1% PVP (w/v) (MW 40,000) (Bangalore Genei). The solution was thoroughly mixed by a vortex mixer (Bangalore Genei). The samples along with DNA extraction buffer were incubated at 65°C for 45 min with slow shaking after 15 min intervals. An equal volume of chloroform: isoamylalcohol (24: 1, v/v) (Himedia) was added to the extract prior to centrifugation (Eppendorf, USA) at 14,000 rpm for 10 min at room temperature (RT). Further, an equal volume of isopropanol (Himedia) and 1/10th volume of sodium acetate (Himedia) was mixed to the supernatant and consequently incubated at 14,000 rpm for 15 min at RT. Supernatant was poured off carefully to avoid loosing the DNA pellet. The pellet was washed with 70% ethanol (Himedia) at 14,000 rpm for 2 min and ethanol was pour off watchfully. Afterwards, the tube was air dried and DNA was dissolved with Tris-EDTA buffer (Himedia). The DNA quality and quantity were evaluated spectrophotometrically at $OD_{260/280\ nm}$ and by visual assessment of band intensities on a 0.8% agarose gel in comparison to Lambda DNA marker (Himedia). Initial screening was done with 40 primers (Operon Technologies Inc., CA, USA) using DNA as template from 5 different landraces. PCR (polymerase chain reaction)-RAPD analysis was repeated at least twice to identify primers that could produce strong and reproducible bands. We observed that among the 40 primers only 10 produced strong and reproducible bands. Accordingly those 10 primers were used in the final analysis for all 28 landraces. Band profiles were scored manually and compiled into a rectangular binary matrix (not shown). Positive amplifications were treated as separate characters and scored for the presence (1) or absence (0) of bands (**Fig. 1**). Only intensely stained, unambiguous bands were scored.

Polymerase chain reaction

DNA amplification was performed in a thermal cycler (BioRad, USA). The PCR reaction was carried out in a final volume of 25 µl reaction mixture which contained 1X PCR buffer (1x *Taq* DNA polymerase buffer (10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mm $MgCl_2$, 0.01% gelatin), 100 µM each of dNTPs (Bangalore Genei), 5 pM primer, 20 ng of genomic DNA and 0.3 U of *Taq* DNA polymerase (Bangalore Genei). PCR was performed with the following temperature regime: An initial one cycle of 5 min at 94°C, followed by 35 cycles each of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. The final extension step was programmed at 72°C for 7 min. Reactions were held indefinitely at 4°C. This procedure was repeated at least twice to check reproducibility. A 20 µl sample of each reaction mixture was then subjected to 1.4% agarose gel electrophoresis and stained with ethidium bromide (Himedia). A 1 kb ladder (Bangalore Genei) was used as the DNA marker and the amplified fragments were visualised under ultra violet (UV) light and recorded on a gel documentation system (BioRad).

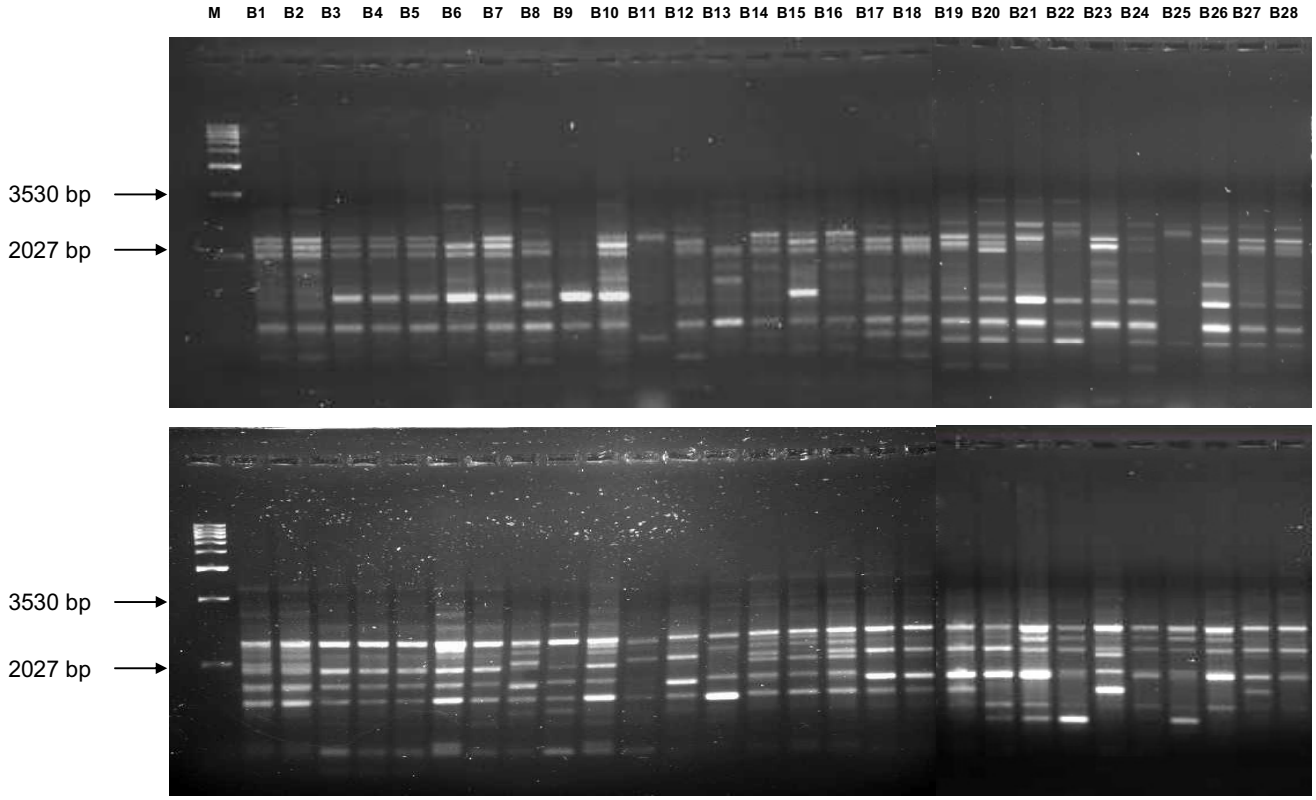

Fig. 1 Amplification of genomic DNA from *Musa* land races with primers OPA 2 (top) and OPA 3 (bottom). M = 1 kb ladder; B1-B28 are different land races mentioned in **Table 1**.

Data analysis

The percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. Although visualization of DNA fragments with different sizes on the agarose gel did not exclude the possibility that some might contain homologous DNA sequences. For the purpose of data analysis individual primer-specific amplification products were considered to represent the dominant allele at a unique RAPD locus.

The degree of polymorphism was quantified by using Shannon's index (Shannon and Weaver 1963) of phenotypic diversity:

$H_0 = - \Sigma \pi_i \, 1n \, \pi_i$

where π_i is the frequency of phenotype i (King and Schaal 1989) and n is the number of populations. Therefore, H_0 can be calculated and compared for different populations.

Let

$H_{pop} = 1/n \, \Sigma \, H_0$ be the average diversity over n different populations and let $H_{sp} = -\Sigma \, \pi 1n \, \pi$ be the diversity calculated from phenotypic frequencies π in all the populations considered together. Then the proportion of diversity present within populations (H_{pop}/H_{sp}) can be compared with that between populations $\{(H_{sp}-H_{pop})/ H_{sp}\}$.

Estimates of similarity between genotypes were based on the probability that an amplified fragment from one accession will also be present in another (Nei and Li 1979).

D_{AB} = 2 X number of shared bands/(number of fragments_A + number of fragments_B)

RESULTS AND DISCUSSION

The PCR-RAPD patterns of DNA extracted from 28 *Musa* landraces were examined. In the beginning of our experiment, 40 10-mer primers (Operon Technologies Inc., CA, USA) from Kits A and E were screened to study the robustness of amplification, reproducibility, and scorability of banding patterns. These primers yielded more than 135 scorable polymorphic bands although only 10 gave reproducible product formation, and these were finally selected for diversity analysis in all landraces. These primers (**Table 3**) generated 66 amplification products, 55 bands (72.7%) of which were polymorphic. The number of bands per primer varied between 3 (OPA 01, OPE 05) and 14 (OPA 02), with an average of 6.6 bands per primer. However, the range of polymorphic bands per primer was 1 (OPE 05, OPE 06) to 14 (OPA 14), with a mean of 5.5 polymorphic bands per primer (**Table 3**). The representative RAPD patterns generated by primers OPA 02 and OPA 03 for 28 landraces are illustrated in **Fig. 1**.

Table 2 Distribution of amplified fragments in *Musa* spp*.

Parameters	Values
Total No. of primer screened	40
Number of primers producing polymorphism	10
Total No. of loci scored	66
Total No. of polymorphic loci	55
Size of amplified band	300 bp – 1 kb
Average No. of polymorphic bands per primer	5.5
Percentage of total bands which are polymorphic	72.7

* Data pooled from studies on 28 individuals from North Bengal and some parts of North Eastern India.

Similar studies have also been conducted by others. For example, Bhat and Jarret (1995) evaluated genetic diversity in 57 accessions of Indian *Musa* by RAPD. They screened 60 random primers but only 49 gave reproducible DNA amplification products. The number of bands per amplification varied from 1 to 24. Uma *et al*. (2006) also studied the interspecific relationship of wild *Musa balbisiana* Colla by RAPD markers. Sixteen accessions of wild banana from different regions of India were collected and 80 primers were initially tested for amplification. However, 34 primers produced reproducible bands, 4 of which gave more than 5 polymorphic bands. In another investigation 21 commercial banana cultivars of South India were examined through RAPD analysis (Venkatachalam *et al.* 2008). In that study out of 75 primers only 50 produced robust amplification products. Similarly, genetic relationships between 20 selected banana cultivars were studied in Kenya by Ongusa *et al*. (2004). In that work 25 random primers were used to observe banding patterns, although only 19 primers were included in the statistical analysis for the purpose of characterization.

Table 3 Number of amplification products generated with most 10 polymorphic primers.

Primer code	Sequence 5′→ 3′	Total № of bands	Polymorphic bands*	Polymorphism % of Sikkim	Polymorphism % of North Bengal	Polymorphism % of Arunachal Pradesh
OPA-01	CAGGCCCTTC	3	2 (66.6)	2	2	1
OPA-02	TGCCGAGCTG	14	14 (100.0)	9	7	3
OPA-03	AGTCAGCCAC	12	11 (91.6)	5	7	2
OPA-13	CAGCACCCAC	5	3 (60.0)	3	3	0
OPE-04	GAGACATGCC	8	8 (100.0)	6	4	3
OPE-05	TCAGGGAGGT	3	1 (33.3)	2	2	0
OPE-06	AAGACCCCTC	4	1 (25.0)	2	3	1
OPE-09	CTTCACCCGA	4	2 (50.0)	3	2	2
OPE-16	GGTGACTGTG	8	8 (100.0)	7	5	1
OPE-20	AACGGTGACC	5	5 (100.0)	4	4	2
Total		66	55(72.7)	43	39	15
Mean per primer		6.6	5.5	65.15%	59.09%	22.73%

*Data in parenthesis represent % of product detecting polymorphism

Table 4 Estimates of genetic diversity (H_0) within three population.

Primers	Sikkim	North Bengal	Arunachal Pradesh
OPA-01	0.43	0.65	0.28
OPA-02	0.80	0.95	0.28
OPA-03	0.60	0.72	0.28
OPA-13	0.66	0.79	0.28
OPE-04	0.44	0.56	0.28
OPE-05	0.54	0.62	0.28
OPE-06	0.47	0.58	0.28
OPE-09	0.41	0.52	0.28
OPE-16	0.65	0.77	0.28
OPE-20	0.56	0.68	0.28
Average	0.55	0.68	0.28

Table 5 Partitioning of genetic diversity into within and between populations for 10 primers.

Primers	H_{sp}	H_{pop}	H_{pop}/H_{sp}	$(H_{sp}-H_{pop})/H_{sp}$
OPA-01	0.85	0.39	0.45	0.54
OPA-02	0.93	0.54	0.58	0.41
OPA-03	0.91	0.48	0.52	0.47
OPA-13	0.86	0.45	0.52	0.47
OPE-04	0.80	0.37	0.46	0.51
OPE-05	0.75	0.42	0.56	0.44
OPE-06	0.85	0.41	0.48	0.51
OPE-09	0.78	0.37	0.47	0.52
OPE-16	0.83	0.48	0.57	0.42
OPE-20	0.79	0.42	0.53	0.46
Average	0.83	0.43	0.51	0.48

Table 6 Similarity Index based on Nei's estimates of similarity of 28 landraces of *Musa* spp.

Landraces																												
B1	1.00																											
B2	1.00	1.00																										
B3	0.68	0.68	1.00																									
B4	0.50	0.50	0.60	1.00																								
B5	0.72	0.72	0.96	0.57	1.00																							
B6	0.66	0.66	0.69	0.43	0.72	1.00																						
B7	0.71	0.71	0.81	0.62	0.78	0.66	1.00																					
B8	0.51	0.51	0.40	0.45	0.39	0.41	0.46	1.00																				
B9	0.41	0.41	0.60	0.45	0.58	0.50	0.62	0.48	1.00																			
B10	0.63	0.63	0.52	0.36	0.55	0.69	0.63	0.47	0.47	1.00																		
B11	0.46	0.46	0.55	0.34	0.53	0.37	0.51	0.37	0.53	0.42	1.00																	
B12	0.61	0.61	0.53	0.50	0.51	0.44	0.55	0.58	0.58	0.45	0.52	1.00																
B13	0.55	0.55	0.53	0.44	0.51	0.48	0.55	0. 46	0.51	0.50	0.41	0.62	1.00															
B14	0.67	0.67	0.60	0.43	0.63	0.59	0.67	0.50	0.45	0.65	0.50	0.53	0.53	1.00														
B15	0.65	0.65	0.54	0.37	0.57	0.62	0.60	0.40	0.36	0.73	0.40	0.46	0.47	0.83	1.00													
B16	0.68	0.68	0.57	0.40	0.60	0.64	0.63	0.47	0.42	0.71	0.47	0.54	0.54	0.93	0.90	1.00												
B17	0.58	0.58	0.56	0.48	0.54	0.51	0.58	0.55	0.55	0.52	0.55	0.72	0.64	0.55	0.50	0.57	1.00											
B18	0.60	0.60	0.58	0.50	0.56	0.48	0.60	0.51	0.57	0.50	0.57	0.75	0.60	0.52	0.47	0.54	0.96	1.00										
B19	0.50	0.50	0.53	0.56	0.51	0.40	0.55	0.51	0.51	0.50	0.51	0.56	0.44	0.48	0.38	0.45	0.64	0.66	1.00									
B20	0.54	0.54	0.48	0.40	0.51	0.48	0.50	0.62	0.51	0.58	0.46	0.50	0.45	0.52	0.47	0.54	0.67	0.64	0.65	1.00								
B21	0.32	0.32	0.35	0.33	0.37	0.33	0.32	0.45	0.40	0.37	0.36	0.34	0.24	0.35	0.28	0.34	0.42	0.40	0.53	0.67	1.00							
B22	0.54	0.54	0.48	0.35	0.51	0.48	0.50	0.46	0.51	0.58	0.51	0.55	0.45	0.52	0.47	0.54	0.67	0.64	0.65	0.80	0.67	1.00						
B23	0.41	0.41	0.48	0.40	0.47	0.41	0.50	0.51	0.46	0.46	0.46	0.45	0.60	0.44	0.36	0.42	0.57	0.54	0.50	0.50	0.40	0.50	1.00					
B24	0.29	0.29	0.36	0.30	0.35	0.37	0.37	0.46	0.46	0.42	0.41	0.44	0.36	0.48	0.43	0.50	0.53	0.50	0.50	0.59	0.53	0.54	0.45	1.00				
B25	0.39	0.39	0.38	0.23	0.37	0.39	0.39	0.40	0.44	0.44	0.55	0.53	0.48	0.47	0.41	0.48	0.62	0.58	0.48	0.58	0.46	0.68	0.48	0.64	1.00			
B26	0.37	0.37	0.40	0.34	0.38	0.37	0.41	0.51	0.51	0.45	0.41	0.55	0.45	0.40	0.35	0.42	0.63	0.60	0.55	0.70	0.63	0.70	0.54	0.71	0.64	1.00		
B27	0.48	0.48	0.51	0.48	0.50	0.43	0.53	0.55	0.55	0.48	0.50	0.66	0.48	0.47	0.41	0.48	0.74	0.76	0.72	0.68	0.51	0.63	0.53	0.64	0.62	0.76	1.00	
B28	0.41	0.41	0.45	0.40	0.43	0.37	0.46	0.48	0.53	0.42	0.48	0.65	0.41	0.41	0.36	0.42	0.66	0.69	0.64	0.67	0.55	0.67	0.46	0.69	0.68	0.83	0.90	1.00
landraces	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21	B22	B23	B24	B25	B26	B27	B28

The number of individual samples considered in this study might not truly represent the total available diversity of *Musa* of this region; nevertheless, the percentage of polymorphic bands (72.7%) (**Table 2**) of RAPD marker in the species was higher than some other plants such as *Changium smyrnioides* (69%) (Fu *et al.* 2003), *Lactoris fernandeziana* (24.5%) (Brauner *et al.* 1992), or *Cathaya argyrophylla* (32%) (Wang *et al*. 1996). This numerical value also suggests that species genetic diversity was high and hence enables it to adapt environmental variations. This study also demonstrated that the Sikkim populations are sufficiently diverse accounting for 65.15% of the total variation observed. The low percentage of variation (59.09 and 22.73%) detected in the North Bengal and Arunachal Pra-

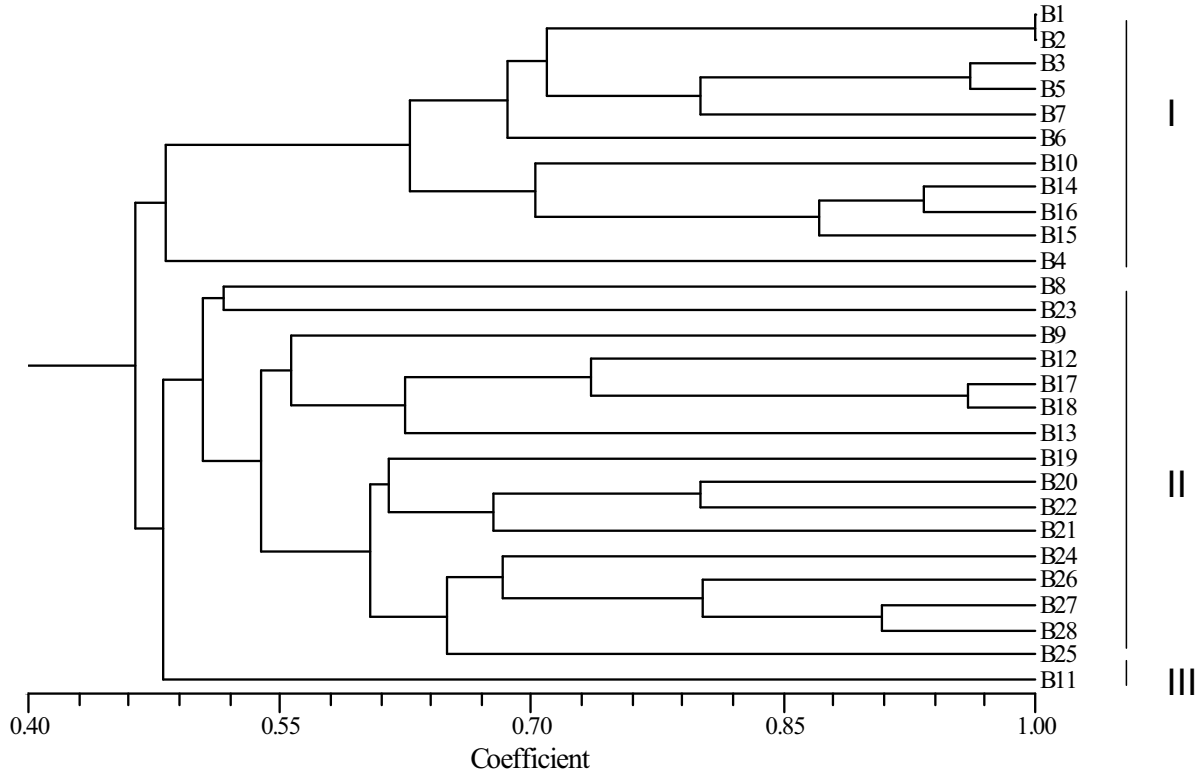

Fig. 2 Dendrogram of different land races of *Musa* based on average linkage cluster analysis.

desh populations, respectively could be attributed to a very limited number of samples (**Table 3**). Although, the reason for lower variation among the Arunachal Pradesh populations is unknown to us at present, yet, it may be attributed to vegetative propagation, which happens to be the only method of reproduction.

The phenotypic frequencies detected within the 10 primers were calculated and used to estimate diversity (H_0) within population types. The population of Arunachal Pradesh exhibited the lowest within-population variability due to the smallest population size which seemed to affect H_0 (**Table 4**). However, both North Bengal and Sikkim populations showed higher variability. Shanon's index of phenotypic diversity was then used to partition the diversity into within and between population components (**Table 5**). On average the proportion of diversity present within population (H_{pop}/H_{sp}) was 51% compared to the between-population diversity $\{(H_{sp}\text{-}H_{pop})/H_{sp})\}$, which was 48%. However, the primers differed in their capacity to detect within and between population variability. Primers OPA-02 and OPA-01 detected the maximum of 58 and 54% within and between population diversity.

Analysis of genetic similarity value (**Table 6**) revealed that – except for B1 and B2 – no two genotypes were identical and that the highest degree of genetic similarity (96%) was recorded in varieties B3, B5 of Cluster I and B17, B18 of Cluster II. Both B1 and B2 genotypes are from the same geographical location (i.e., East Sikkim), which may be a potential reason for such a high degree of similarity and a typical case of duplicate entries. On the other hand, B4 (Sikkim) and B25 (North Bengal) showed a very low similarity value of 23%, (i.e., high degree of dissimilarity) which is in broad agreement with the geographical distribution of these two genotypes. Moreover, this can be attributed to the broad genetic base in the origin of the species. This similarity coefficient values of banana in this study are higher or in the same range with respect to other reported species such as *Panax ginseng* (19.7-49.1%) (Um *et al.* 2001), *Poa trivialis* (7-74%) (Rajasekar *et al*. 2006), *Rhododendron* sp (26.2-90.6%) (Lanying *et al*. 2008), *Lathyrus sativus* (13-66%) (Sedehi *et al*. 2008), common bean (19-91%) (Tiwari *et al*. 2005), *Ensete ventricosum* (16-85%) (Birmeta *et al*. 2002).

The degree of polymorphism revealed in 3 populations by amplification with arbitrary primers is extensive. The degree of polymorphism in this study may be due to the wide geographical origin of the genotypes. A dendrogram constructed on the basis of shared fragments revealed the broad existence of three clusters (**Fig. 2**). Cluster I or Sikkim and Arunachal Pradesh was linked to cluster II or North Bengal at low similarity coefficient of 53%. In general the clustering concurs with the place of collection of different genotypes. It also reveals that B1 and B2 are same genotypes as revealed by their similarity coefficient as 100%. Essentially little morphological variation between them misled us to treat them as different landraces. On the contrary, the genotype B11 alone made a separate cluster III.

CONCLUSIONS

The present study addresses the utility of RAPD markers in revealing genetic relationships at molecular level among landraces of *Musa* spp. of North Bengal as well as North Eastern part of India. The RAPD polymorphism may be attributed to the outcome of a nucleotide change that alters the prime-binding site or an insertion or deletion within the amplified region (Willams *et al*. 1993). The RAPD markers were able to distinguish groups among the banana cultivars in different clusters. The polymorphism revealed by RAPD has been problematic due to their dominance. As heterozygotes are not normally detectable, the results are not readily usable for computing Hardy-Weinberg equilibrium or Nei's standard genetic distance (Lynch and Milligan 1994). The level of polymorphism observed in the present study was moderately high, indicating a wide and diverse genetic base for the banana landraces in North-Eastern part of India. The 72.7% RAPD polymorphic bands suggest that banana landraces maintain a higher intra-specific genetic diversity of North-East India. The conventional classification of banana genotypes into distinct genome combinations is based on their morphological similarity to *M. acuminata* Colla or *M. balisiana* Colla. The landraces which were tested in this study did not cluster accordingly to their hypothetical genetic homologies. However, cultivars with two

different genotypes (Chinichampa AB and Malbhog AAB) clustered together. The difficulty faced in the identification of banana cultivars, which are mostly sterile, therefore highlights the need for a DNA marker system for classification (Loh *et al*. 2000). The DNA fingerprinting pattern would help in the identification of duplications among accessions in the field.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. C. P. Suresh and Dr. Pralad Dev, Department of Post Harvest and Pomology for helping to collect the sample and Department of Food Processing Industries, Govt of West Bengal for financial assistance and Mr. Kamal Das of this laboratory for his assistance.

REFERENCES

Agoreyo BO, Golden KD, Brown SE (2008) Analysis of genetic variability among plantain cultivars (*Musa paradisiaca* L.) using arbitrarily primed PCR technique. *African Journal of Biotechnology* **7**, 1041-1045

Bhat KV, Jarret RL (1995) Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. *Genetic Resources and Crop Evolution* **42**, 107-118

Bhat KV, Jarret RL, Rana RS (1995) DNA profiling of banana and plantain cultivars using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. *Electrophoresis* **16**, 1736-1745

Birmeta G, Nybom H, Bekele E (2002) RAPD analysis of genetic diversity among clones of the Ethiopian crop plant *Ensete ventricosum*. *Euphytica* **124**, 315-325

Brauner S, Crawford DJ, Stuessy TF (1992) Ribosomal DNA and RAPD variation in the rare plant family Lactoridaceae. *American Journal of Botany* **79**, 1436-1439

Cheesman EE (1947) Classification of bananas. II. The genus *Musa* L. *Kew Bulletin* **2**, 106-117

De Langhe EA (2000) Diversity in the genus *Musa*: its significance and its potential. *Acta Horticultarae* **540**, 81-88

Fu C, Qiu Y, Kong H (2003) RAPD analysis for genetic diversity in *Changium smyrnioides* (Apeaceae), and endangered plant. *Botanical Bulletin of Academia Sinica* **44**, 13-18

Ge XJ, Liu MH, Wang WK, Schaal BA, Chiang TY (2005) Population structure of wild bananas, *Musa balbisiana*, in China determined by SSR fingerprinting and cpDNA PCR-RFLP. *Molecular Ecology* **14**, 933-944

Howell EC, Newbury HJ, Swennen RL, Withers LA, Ford-Lloyd BV (1994) The use of RAPD for identifying and classifying *Musa* germplasm. *Genome* **37**, 328-332

Jarret RL, Gawel NJ, Wittermore A (1992) RFLP-based phylogeny of *Musa* species in Papua New Guinea. *Theoretical and Applied Genetics* **84**, 579-584

Kaemmer D, Afza R, Weisang K, Kahl G, Novak FJ (1993) Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (*Musa* ssp.). *Biotechnology* **10**, 1030-1035

King LM, Schaal BA (1989) Ribosomal DNA variation and distribution in *Rudbeckia missouriensis*. *Evolution* **43**, 1117-1119

Lanying Z, Yongqing W, Li Z (2008) Genetic diversity and relationship of *Rhododendron* species based on RAPD analysis. *American-Eurasian Journal of Agriculture and Environmental Science* **3**, 626-631

Loh JP, Kiew R, Set O, Gan LH, Gan YY (2000) Amplified fragment length polymorphism fingerprinting of 16 banana cultivars (*Musa* cvs.). *Molecular Phylogenetics and Evolution* **17**, 360-366

Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology* **3**, 91-99

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences USA* **76**, 5269-5273

Onguso JM, Kahangi EM, Ndiritu DW, Mizutani F (2004) Genetic characterization of cultivated bananas and plantains in Kenya by RAPD markers. *Scientia Horticulturae* **99**, 9-20

Pillay M, Ogundiwin E, Nwakanma DC, Ude G (2001) Analysis of genetic diversity and relationships in East African banana germplasm. *Theoretical and Applied Genetics* **102**, 965-970

Rajasekar S, Fei S, Christians NE (2006) Analysis of genetic diversity in rough bluegrass determined by RAPD markers. *Crop Science* **46**, 162-167

Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1984) Extraordinary polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and population dynamics. *Proceedings of the National Academy of Sciences USA* **91**, 5466-5470

Sedehi AAV, Solooki M, Arzani A, Ghanbari A, Lotfi A, Imamjomeh AA, Bahrami S (2008) Comparative analysis of genetic diversity among grass pea landraces as detected by random, semi random and morphological markers. *Asian Journal of Plant Sciences* **7**, 454-460

Shannon CE, Weaver W (1963) *The Mathematical Theory of Communication*, University Illinois Press, Urbana, 117 pp

Simmonds NW (1995) Bananas: *Musa* (*Musaceae*). In: Smartt J, Simmonds NW (Eds) *Evolution of Crop Plants* (2nd Edn), Longmans Scientific and Technical, Essex, UK, pp 370-375

Song ZP, Xu X, Wang B, Chen JK, Lu BR (2003) Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. *Theoretical and Applied Genetics* **107**, 1492-1499

Stover RH, Simmonds NW (1987) *Bananas* (3rd Edn), Longman Scientific and Technical, Essex, England, pp 1-20

Tandon P, Kumaria S, Nongrum L (2009) Conservation and management of plant genetic resources of Northeast India. *Indian Journal of Traditional Knowledge* **8**, 29-34

Tiwari M, Singh NK, Rathore M, Kumar N (2005) RAPD markers in the analysis of genetic diversity among common bean germplasm from central Himalaya. *Genetic Resources and Crop Evolution* **52**, 315-324

Ude G Pillay M, Nwakanma D, Tenkouano A (2002) Genetic diversity in *Musa acuminata* Colla and *Musa balbisiana* Colla and some of their natural hybrids using AFLP markers. *Theoretical and Applied Genetics* **104**, 1246-1252

Um JY, Chung HS, Kim MS, Na HJ, Kwon HJ, Kim JJ, Lee KM, Lee SJ, Lim JP, Do KR, Hwang WJ, Lyu YS, An NH, Kim HM (2001) Molecular authentication of *Panax ginseng* species by RAPD analysis and PCR-RFLP. *Biological Pharmacology Bulletin* **24**, 872-875

Uma S, Siva SA, Saraswathi MS, Durai P, Sharma TVRS, Singh DB, Selvarajan R, Sathiamoorthy S (2005) Studies on the origin and diversification of Indian wild banana (*Musa balbisiana*) using arbitrarily amplified DNA marker. *Journal of Horticultural Science and Biotechnology* **80**, 575-580

Uma S, Siva SA, Saraswathi MS, Manickavasagam M, Durai P, Selvarajan R, Sathiamoorthy S (2006) Variation and intraspecific relationships in Indian wild *Musa balbisiana* (BB) population as evidenced by random amplified polymorphic DNA. *Genetic Resources and Crop Evolution* **53**, 349-355

Venkatachalam L, Sreedhar RV, Bhagyalakshmi N (2008) The use of genetic markers for detecting DNA polymorphism, genotype identification and phylogenetic relationships among banana cultivars. *Molecular Phylogenetics and Evolution* **47**, 974-985

Virk PS, Newbury HJ, Jackson MT, Ford-Lloyd BV (2000) Are mapped or anonymous markers more useful for assessing genetic diversity? *Theoretical and Applied Genetics* **100**, 607-613

Wang XQ, Zou YP, Zhang DM Hong DY (1996) RAPD analysis for genetic polymorphism in *Cathaya argyrophylla*. *Science in China* **26**, 437-441

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers as useful genetic markers. *Nucleic Acids Research* **18**, 6531-6535

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV (1993) Genetic analysis using random amplified polymorphic DNA markers. *Methods in Enzymology* **218**, 704-740

Wong C, Kiew R, Argent G, Set O, Lee SK, Gan YY (2002) Assessment of the validity of the sections in *Musa* (Musaceae) using AFLP. *Annals of Botany* **90**, 231-238