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# Genetic diversity study of some banana genotypes collected from various parts of India through RAPD analysis

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Globally, banana is the fourth most important fruit crop and is grown in more than 130 countries across the world. Traditional procedure to characterize banana plants by morphological descriptors has many limitations, and different communities refer to the same local cultivars by different names due to lack of clear clone identity resulting in unnecessary duplication with regard to cultivation, conservation and research. To alleviate this problem application of modern finger printing technology through DNA studies have been recommended for accurate selection of banana clones. Here, 16 genotypes of banana collected from different districts of West Bengal and other parts of India were characterized by RAPD analysis to assist the selection of characters for banana breeding. It was observed that among the 25 randomly selected markers, OPE 1, 4, 7, 9, 20 and 25 showed 100% polymorphism under annealing temperatures of 27 and 29°C. Their genetic diversity study revealed that sixteen germplasms were grouped into eight clusters namely Malbhog, Martaman, Kalibhog and Sobri in Cluster-I; Alapan, Poovan and Champa in Cluster-II; Amritpani, Bamandeshi in Cluster-III; Dudhsagar, Rasthali in Cluster-IV; Krishna Vazai, Manohar in Cluster-V; Chang Monua in Cluster VI; Kanai Bansi in Cluster VII and Nendran in Cluster VIII.

**Key words:** Banana, molecular characterization, genetic diversity.

## INTRODUCTION

Banana (*Musa* sp.) is the maximum distributed fruit crop and is the fourth most important commodity in the world. It is grown in more than 130 countries across the world, continuously exhibiting a spectacular growth pattern worldwide, producing 120 million tonnes (Anonymous,

2014) of which 56% is shared by Asia. India is the largest producer of banana in the world with the production of 29.72 million tonnes during 2013-14 (Anonymous, 2015). There are diverse germplasms of banana traditionally cultivated in different regions of India having remarkable

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genetic differences, in spite of their overall genomic grouping variations still existing in the same group. Despite the importance of bananas in trade and commerce, there is little information on the genetics for its agronomically important traits (Loh et al., 2000). Traditional procedure to characterize banana plants by morphological descriptors has many limitations. Many improved varieties released have a complex genealogy involving several wild species and landraces. However, barriers such as intractable fertilization, moderate to high levels of female sterility and triploidy have made the identification of desired banana cultivars a key issue for these crop improvement programmes (Bhat et al., 1995). In view of developing efficient breeding schemes, additional data needs to be generated on the complex genome structure of hybrids and cultivars. To this end, the characterization of indigenous germplasms will offer a precise means of formulating taxonomic, phylogenetic and heterotic groupings within the family Musaceae (Crouch et al., 1998).

Cheesman (1948) first suggested that cultivated bananas originated from intra and interspecific hybridization between the two wild diploid species *Musa acuminata* Colla and *Musa balbisiana* Colla, each contributing the A and B genomes, respectively. The identification of *Musa* cultivars has traditionally been based upon various combinations of morphological, phenological and floral criteria. Simmonds and Shepherd (1955) devised a scoring technique based on 15 diagnostic morphological characters to differentiate *M. acuminata* clones from *M. balbisiana* cultivars and their hybrids into 6 genome groups. The taxonomy of cultivated bananas has long been a contentious issue and because it relies heavily on morphology, the literature shows many contradictions. For instance, based on molecular data, Pillay et al. (2000) showed that the clones 'Monthan Saba' and 'Bluggoe', previously classified as BBB based on morphological characteristics but, actually belong to the ABB group. Similarly, tetraploid 'KlueTiparot' (ABBB) is reclassified as a triploid ABB (Jenny and Carreel, 1997; Horry et al., 1998). The difficulties associated with the use of whole plant or floral morphology has led researchers to develop other techniques for the correct identification of *Musa* species and cultivars.

Different communities refer to the same local cultivars by different names, and lack of clear clone identity in the crop has resulted in unnecessary duplication in cultivation, conservation and research (Onguso et al., 2004). To alleviate this problem, application of modern finger printing technology through DNA studies have been recommended for accurate selection of banana clones (Robinson, 1996).

As a more reliable alternative, various DNA fingerprinting techniques have been used to study the genetic diversity and taxonomy of cultivated bananas which include disozyme analysis (Bhat et al., 1992),

restriction fragment length polymorphism (RFLP) (Bhat et al., 1994; Jarret et al., 1992; Kaemmer et al., 1992), rRNA spacer length heterogeneity (Lanaud et al., 1992), inter simple sequence repeat (ISSR) markers (Godwin et al., 1997), sequence tagged microsatellite sites (STMS) (Grapin et al., 1998; Kaemmer et al., 1997) and amplified fragment length polymorphism (AFLP) (Loh et al., 2000; Wong et al., 2001).

To make the collection useful for plant breeders, morphological and molecular characterization of the germplasms is necessary. The disadvantages of phenotype-based assays can be overcome by direct identification of genotypes with DNA-based markers (Nsabimana and Staden, 2007). Molecular markers have been employed in *Musa* genotypes to assess ploidy (Oselebe et al., 2006), phylogenetic relationships (Jain et al., 2007; Nsabimana and Staden, 2007; Uma et al., 2006) and genetic diversity because of somaclonal variation (Lakshmanan et al., 2007; Bairu et al., 2006; Ray et al., 2006) or mutation induction (Hautea et al., 2004; Finalet et al., 2000; Toruan-Mathius and Haris, 1999). Polymorphisms generated by RAPD analysis has been used for fingerprinting and classification of the *Musa* genotypes. Linkage of RAPD markers to specific traits such as disease resistance has been possible (Damasco et al., 1996) and RAPD markers are usually preferred as the technique is simple, versatile, relatively inexpensive and able to detect minute differences (Pillay et al., 2000; Williams et al., 1990; Welsh and McClelland, 1990; Howell et al., 1994). RAPD based fingerprinting has been successfully applied to the characterization of diverse *Musa* germplasms (Bhat and Jarret, 1995; Onguso et al., 2004), analysis of *Musa* breeding populations (Crouch et al., 1999) and detection of somaclonal variants (Grajal-Martin et al., 1998). A proper classification of *Musa* clones and cultivars is important in assisting the selection of characters for banana breeding. This research work therefore describes the use of RAPD markers to evaluate the genetic diversity and relationships amongst sixteen different Mauritian *Musa* germplasms collected from different parts of India.

## MATERIALS AND METHODS

### Plant materials

The present study was made at the Department of Fruits and Orchard Management, Faculty of Horticulture, Bidhan Chandra KrishiViswavidyalaya, Nadia, West Bengal, India. Sixteen germplasms of banana collected from various states of India (Table 1) were studied for their genetic diversity and relationship. Young leaves from nursery grown randomly selected plant as well as its donor plants, were taken for genomic DNA isolation.

### Plant DNA isolation

Fresh, green tender leaf samples were collected from the field and were immediately wrapped in aluminum foil and stored until the

**Table 1.** Banana genotypes used in the present study.

Germplasm	Genome	Sub group	Source
Sobri	AAB	Silk	Krishnaganj, Majhdia, Nadia, West Bengal
Nendran	AAB	Plantain	BRS, Kannara, Kerala
Krishnavazai	AAB	Pome	Horticultural Research and Developmental Farm, Chunchura, West Bengal
Malbhog	AAB	Silk	Pundibari, Coochbehar, West Bengal
Rasthali	AAB	Silk	TNAU, Tamil Nadu
Amritpani	AAB	Silk	ARS, Kovvur, Andhra Pradesh
Champa	AAB	Mysore	Bandel, Hooghly, West Bengal
Kalibhog	AAB	Silk	Horticulture Research Farm, Kalyani, West Bengal
Dudhsagar	AAB	Silk	AAU, Jorhat, Assam
Martaman	AAB	Silk	Ghoragancha, Nadia, West Bengal
Kanaibashi	AAB	Hatidat	Chakdaha, Nadia, West Bengal
Chang Monua	AAB	Silk	Baneswar, Coochbehar, West Bengal
Poovan	AAB	Mysore	TNAU, Tamil Nadu
Manohar	AAB	Athiakol	AAU, Jorhat, Assam
Bamandeshi	AAB	Pome	Senpara, Jalpaiguri, West Bengal
Alapan	AAB	Mysore	RAU, Pusa, Bihar

material reached the laboratory. The samples were washed thoroughly and dried with tissue paper. Approximately, 100 mg of laminae (with partially intact petioles) was taken and immediately transfer to precooled (-50°C) mortar following excision from the plant and the tissue stored at -50°C for at least 30 min. The leaf tissue was grinded as quickly as possible and DNA was extracted following the standard procedure of CTAB method with slight modification. The samples were then preserved in a refrigerator (4°C) and quantified accordingly.

#### Optimization of PCR primers

RAPD amplification was performed in 25 µl of reaction mixture that contained template DNA 2.0 µl, 10x PCR buffer 2.5 µl, 2.5 mM dNTPs 2.0 µl, primer 1.0 µl, 2.5 mM MgCl<sub>2</sub> 2.0 µl, Taq polymerase enzyme 0.3 µl, and ddH<sub>2</sub>O 13.0 µl. The PCR reaction mixture of 25 µl was then set for the reaction in PCR machine. The amplification was performed in a thermal cycler (Mastercycler, Eppendorf, AG22331, Germany). The gel was photographed under UV light transillumination (Gel Doc, Biotech, Yercaud, Salem, India). The 20 ng DNA sample and 30 µl polymerase enzyme was used.

#### Screening of primers

Altogether, 25 random decamer oligonucleotide primers (Eurofins Genomics, Bengaluru, India) were screened for the study (Table 2).

#### Agarose gel preparation and gel electrophoresis

Agarose (SRL, India, cat# 01441Q) was dissolved in 100 ml of IX TAE buffer which was then boiled in microwave oven (Godrej, GMG22B) for 3 min and allowed to cool to 60°C and solidified on gel tray (Genei™). In each PCR tube, 2.5 µl of 10x gel loading dye was added. The tube was spinned for 3 s in a centrifuge (Mastercycler, Eppendorf, AG22331, Germany) and loaded onto the agarose gel. The gel was run at constant 80 volts till the dye front moved a distance of about 2/3 from the loading point. The

**Table 2.** Primers used during the study, their sequence and annealing temperature.

S/N	Code	Primer sequence (5'-3')
1	OPE 01	CCAGATGCAC
2	OPE 02	GAGACATGCC
3	OPE 03	AAGACCCCTC
4	OPE 04	CTTCACCCGA
5	OPE 05	TTATCGCCCC
6	OPE 06	GGTGACTGTG
7	OPE 07	GGACTGCAGA
8	OPE 08	ACGGCGTATG
9	OPE 09	AACGGTGACC
10	OPE 10	CCAAGCTTCC
11	OPE 11	CCAGTACTCC
12	OPE 12	TGAGCGGACA
13	OPE 13	CAGGCCCTTC
14	OPE 14	TGCCGAGCTG
15	OPE 15	AGTCAGCCAC
16	OPE 16	AATCGGGCTG
17	OPE 17	AGGGGTCTTG
18	OPE 18	GGTCCCTGAC
19	OPE 19	GTGACGTAGG
20	OPE 20	GTGATCGCAG
21	OPE 21	CAATCGCCGT
22	OPE 22	TCGGCGATAG
23	OPE 23	CAGCACCCAC
24	OPE 24	TTCCGAACCC
25	OPE 25	AGGTGACCGT

Annealing temperature (°C) = 27; 29°C for OPE 13, 14, 18 and 23.

photograph was taken in Geldoc (Biotech, Yercaud, Salem, India).

**Table 3.** Production of total and polymorphic bands using specific primers.

Primer	Number of RAPD products (bands)		
	Total bands	Polymorphic	% polymorphism
OPE 01	2	2	100.0
OPE 02	5	2	40.0
OPE 03	0	0	0
OPE 04	3	3	100.0
OPE 05	0	0	0
OPE 06	2	1	50.0
OPE 07	3	3	100.0
OPE 08	0	0	0
OPE 09	3	3	100.0
OPE 10	0	0	0
OPE 11	0	0	0
OPE 12	4	2	50.0
OPE 13	0	0	0
OPE 14	3	2	66.67
OPE 15	0	0	0
OPE 16	5	4	80.0
OPE 17	0	0	0
OPE 18	0	0	0
OPE 19	5	3	60.0
OPE 20	3	3	100.0
OPE 21	3	2	66.67
OPE 22	0	0	0
OPE 23	0	0	0
OPE 24	4	3	75.0
OPE 25	1	1	100.0

1% agarose gel was used to perform this operation.

#### Statistical analysis

Cluster analysis was done to identify a smaller number of groups such that the genotypes residing in a particular group were more similar to each other than to genotypes belonging to other groups (Singh and Chowdhury, 1985). Grouping in the present study was done by Tocher method (Rao, 1952) with the help of Mahalanobis (1936) method. The dendrogram was generated using unweighted pair group arithmetic mean method (UPGMA) using NTSYS pc version 2.1 software for classifying under several clusters.

## RESULTS AND DISCUSSION

#### Evaluation of parameters for PCR

Initially, three different parameters namely, Taq DNA polymerase concentration, MgCl<sub>2</sub> concentration and primer concentration were examined with fixed amount of *Musa* spp. template DNA (2.0 µl). Optimum concentration was selected based on clear banding pattern. It was observed that a combination of 1.0 µl of primer and 0.3 µl

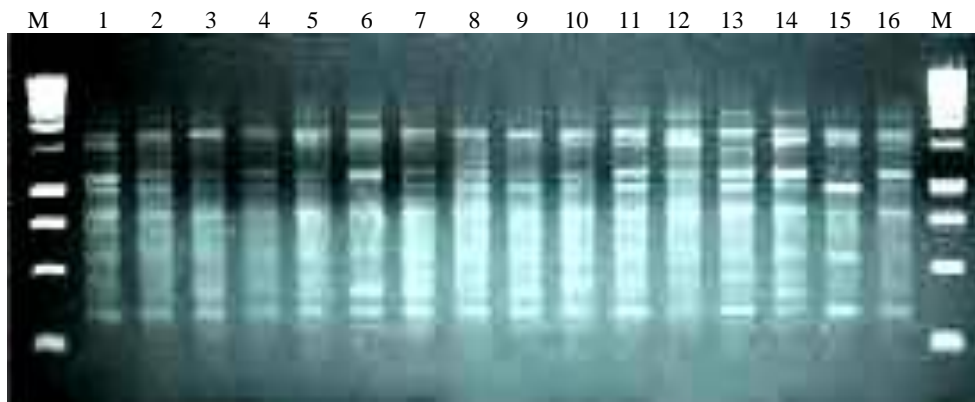
of Taq DNA polymerase gave the best results with addition of 2.0 µl MgCl<sub>2</sub> (25 mM) in the reaction mixture which improved the banding pattern.

#### Annealing temperature

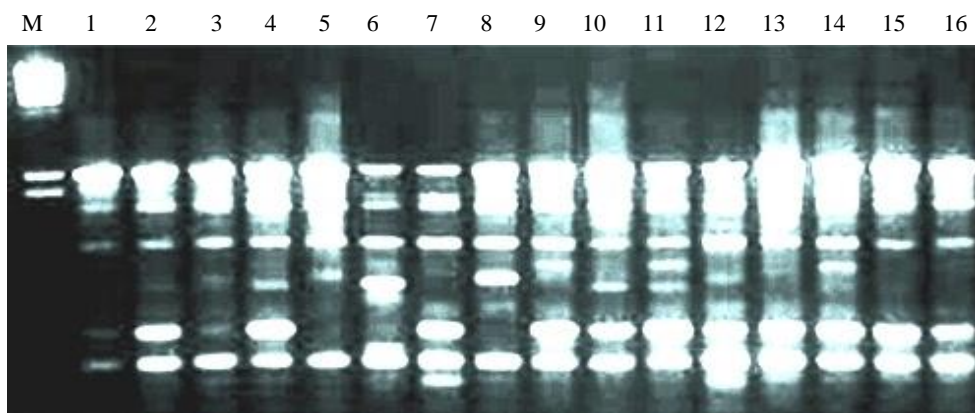
In a separate set of experiments, a regime of two different annealing temperatures (27 and 29°C) were tested (Table 2) with four standardized concentration primers (OPE-01, OPE-02, OPE-04 and OPE-07) and Taq DNA polymerase with cultivars of *Musa* spp. A clear and prominent banding pattern was recorded at these two different annealing temperatures (Table 3), and it was observed that OPE 1, 4, 7, 9, 20 and 25 showed 100% polymorphism.

#### Template DNA

For optimization of template DNA, concentrations such as 1.0, 2.0 and 3.0 µl reaction mixtures were used. Among the different reaction mixtures, 2.0 µl performed best



**Figure 1.** Banding pattern of banana germplasms with primer OPE-02. Lane 1-16: RAPD profile of banana varieties (1) Sobri (2) Nendran (3) Krishna Vazai (4) Malbhog (5) Rasthali (6) Amritpani (7) Champa (8) Kalibhog (9) Dudhsagar (10) Martaman (11) Kanai Bansi (12) Chang Monua (13) Poovan (14) Manohar (15) Bamandeshi (16) Alapan. Lane M: DNA ladder.



**Figure 2.** Banding pattern of banana germplasms with primer OPE-19. Lane 1-16: RAPD profile of banana varieties (1) Sobri (2) Nendran (3) Krishna Vazai (4) Malbhog (5) Rasthali (6) Amritpani (7) Champa (8) Kalibhog (9) Dudhsagar (10) Martaman (11) Kanai Bansi (12) Chang Monua (13) Poovan (14) Manohar (15) Bamandeshi (16) Alapan. Lane M: DNA ladder.

amplification. Lower or higher concentrations either reduced amplification or produced smearing. Therefore, in the subsequent experiments 2.0  $\mu$ l template DNA concentration was used. In all the aforementioned reactions, a control was set up, which contained all the constituents of reaction mixture except template DNA that was replaced by the exact amount of  $sdH_2O$ .

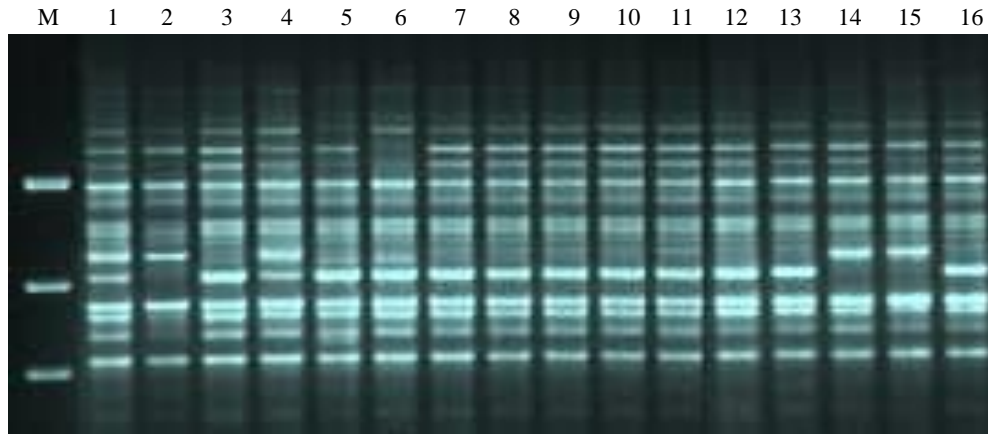
### Screening of primers

As mentioned earlier, 25 random decamer (10 base pair) oligonucleotide sequences (primers) were screened for sixteen germplasms of banana to study the robustness of amplification, reproducibility, and scorability of banding

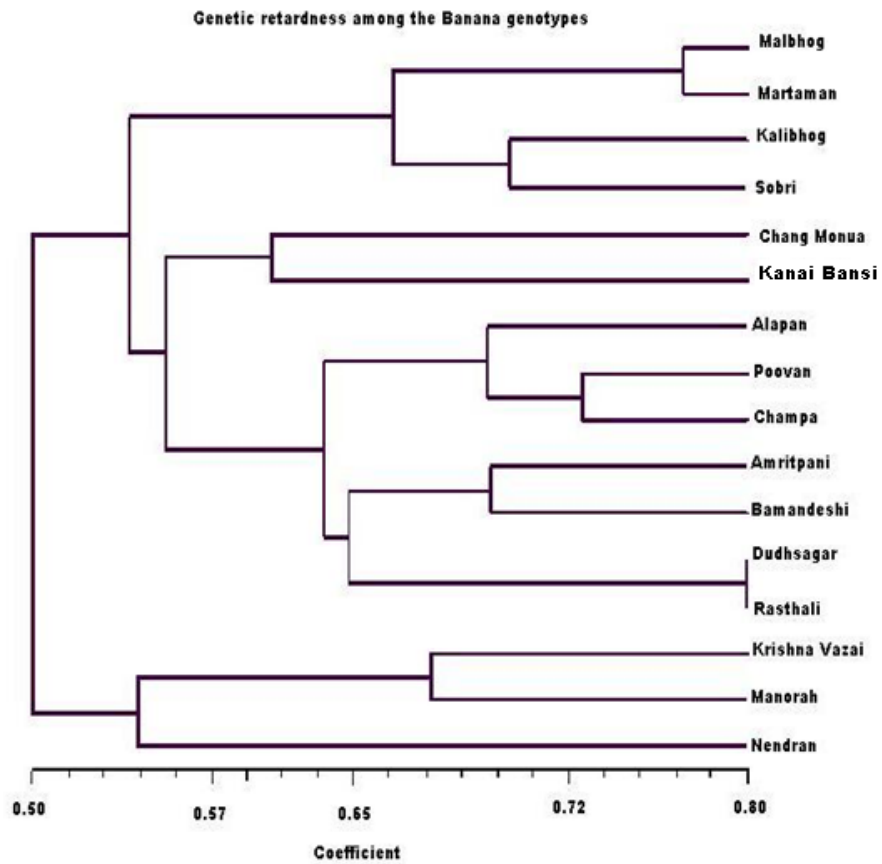
patterns. As a result from the 25 primers, 11 did not produce any polymorphic bands and 1 gave only one band. The RAPD primers showed that higher degree of polymorphism (Table 3 and Figures 1 to 3) were used to identify the genotypes under study and those showing no polymorphism were not suitable for study purpose.

### Grouping of varieties into cluster

The dendrogram (Figure 4) was generated using unweighted pair group arithmetic mean method (UPGMA) using NTSYS pc version 2.1 software and resulted in eight major clusters. From the clustering pattern of the genotypes, it revealed that the 16 genotypes were



**Figure 3.** Banding pattern of banana germplasms with primer OPE-14. Lane 1-16: RAPD profile of banana varieties (1) Sobri (2) Nendran (3) Krishna Vazai (4) Malbhog (5) Rasthali (6) Amritpani (7) Champa (8) Kalibhog (9) Dudhsagar (10) Martaman (11) Kanai Bansi (12) Chang Monua (13) Poovan (14) Manohar (15) Bamandeshi (16) Alapan. Lane M: DNA ladder.



**Figure 4.** Genetic relationships among the 16 banana genotypes under study.

grouped into eight clusters that is, Malbhog, Martaman, Kalibhog and Sobri- Cluster-I; Alapan, Poovan and

Champa- Cluster II; Amritpani, Bamandeshi- Cluster III; Dudhsagar, Rasthali- Cluster IV; Krishna Vazai, Manohar- Cluster V; Chang Monua- Cluster VI; Kanai Bansi- Cluster VII and Nendran- Cluster VIII.

### Analysis of polymorphism

The 25 random 10-mer primers yielded more than 327 scorable polymorphisms. However, the 10 decamer oligonucleotide primers produced reproducible bands (Table 3). They generated 46 amplification products of which 34 bands (73.91%) were polymorphic. The number of bands per primer varied between 1 to 5, with an average of 3.29 bands per primer. However, the range of polymorphic bands per primer was 1 to 4, with a mean of 2.43 polymorphic bands per primer (Table 3). The representative RAPD patterns generated by primers OPE-01, OPE-02, OPE-04, OPE-06, OPE-07, OPE-09, OPE-12, OPE-14, OPE-16, OPE-19, OPE-20, OPE-21, OPE-24 and OPE-25 are illustrated in this research article.

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 (73.91%) 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), *Cathaya argyrophylla* (32%) (Wang et al., 1996). The numerical value also suggested that the species genetic diversity was high and hence enables it to adapt to environmental variations.

The isolates of banana were subjected to PCR analysis where 14 universal primers resulted in robust and reproducible DNA fragment patterns. The selected primers generated numerous bands but, 327 distinct and reproducible were considered for analysis. Each primer showed polymorphic banding pattern. The genetic similarity between the isolates of banana was determined on the basis of Jaccard's similarity coefficient. The highest genetic similarity was observed between Dudhsagar and Rasthali isolates, ranging from 80%. Malbhog and Martaman showed 77% similarity, whereas Poovan and Champa depicted high level of similarity i.e., 74%. Amritpani and Bamandeshi showed 69% similarity. Krishna Vazai and Manohar showed 67% similarity which may be a potential of such a high degree of similarity. There was 65% similarity between the two sub clusters Malbhog and Martaman (familiar in West Bengal and also originated here) and Kalibhog and Sobri (also collected from West Bengal). There was 60% similarity depicted between Chang Monua and Kanai Bansi, both of them made a sub cluster (Figure 4). Kanai Bansi and Chang Monua gave just 60% similarity. The lowest degree of similarity was shown between Nendran and Chang Monua i.e. 50% (high degree of dissimilarity) which is in

broad agreement with the geographical distribution of these two genotypes (These two were collected from various location, Nendran from Kerala and Chang Monua were collected from West Bengal) and 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 is higher or in the same range with respect to other reported species such as *Panax ginseng* (19.7 to 49.1%) (Um et al., 2001), *Poa trivialis* (7 to 74%) (Rajasekar et al., 2006), *Rhododendron* spp. (26.2-90.6%) (Lanying et al., 2008), *Lathyrus sativus* (13-66%) (Sedehi et al., 2008), Common bean (19 to 91%) (Tiwari et al., 2005), *Ensete ventricosum* (16 to 85%) (Birmeta et al., 2002).

The degree of polymorphism revealed in populations by amplification with arbitrary primers is extensive. The degree of polymorphism in this study might be due to the wide geographical origin of the genotypes. A dendrogram constructed based on shared fragments revealed broad existence of clusters. In general, the clustering concurs with the place of collection of different genotypes. It can also be opined that Dudhsagar and Rasthali are same genotypes as revealed by their maximum similarity coefficient. Essentially little morphological variation between them misleads us to treat them as different landraces.

The present study addressed the utility of RAPD markers in revealing genetic relationships at molecular level among landraces of *Musa* spp. of North Bengal and North Eastern, Southern and 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 (Williams et al., 1993). The RAPD markers were able to distinguish groups among the banana cultivars in different clusters. The polymorphism showed 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 (Table 4) was moderately high, indicating a wide and diverse genetic base for the banana landraces in various parts of India. The 73.91% RAPD polymorphic bands suggest that banana landraces maintain a higher intra-specific genetic diversity which is very important for future breeding programme to generate good quality germplasms in the context of climate change and environmental hazards (Maji and Das, 2008; Maji, 2013; Singh et al., 2013; Soni et al., 2013a, b; Kumar et al., 2013). The conventional classification of banana genotypes into distinct genome combinations based on their morphological similarity is as follows *Musa acuminata* Colla or *Musa balbisiana* Colla. 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

**Table 4.** Similarity index based on Nei's estimates of 16 cultivars of *Musa* spp.

	Sobri	Nendran	Krishna Vazai	Malbhog	Rasthali	Amritpani	Champa	Kalibhog	Dudhsagar	Martaman	Kanai Bansi	Chang Monua	Poovan	Manohar	Bamandesi	Alapan
Sobri	1															
Nendran	0.77	1														
Krishna Vazai	0.61	0.7	1													
Malbhog	0.61	0.55	0.6	1												
Rasthali	0.42	0.48	0.52	0.52	1											
Amritpani	0.48	0.62	0.52	0.52	0.6	1										
Champa	0.5	0.56	0.54	0.6	0.54	0.6	1									
Kalibhog	0.63	0.58	0.5	0.56	0.56	0.56	0.68	1								
Dudhsagar	0.54	0.48	0.59	0.67	0.52	0.46	0.65	0.68	1							
Martaman	0.62	0.63	0.61	0.48	0.61	0.54	0.67	0.69	0.6	1						
Kanai Bansi	0.58	0.71	0.7	0.65	0.55	0.62	0.68	0.54	0.57	0.56	1					
Chang Monua	0.41	0.46	0.57	0.57	0.65	0.5	0.64	0.6	0.8	0.65	0.52	1				
Poovan	0.56	0.5	0.54	0.61	0.69	0.54	0.67	0.63	0.6	0.62	0.52	0.52	1			
Manohar	0.45	0.45	0.6	0.54	0.54	0.48	0.71	0.57	0.65	0.61	0.56	0.64	0.74	1		
Bamandesi	0.46	0.39	0.36	0.67	0.5	0.58	0.52	0.48	0.5	0.4	0.48	0.41	0.59	0.46	1	
Alapan	0.44	0.38	0.41	0.48	0.48	0.41	0.44	0.41	0.48	0.38	0.46	0.33	0.71	0.56	0.61	1

DNA fingerprinting pattern would help in the identification of duplications among accessions in the field.

## Conclusions

The present study clearly concluded that there was a wide genetic variation in the banana germplasms collected from various parts of India. 14 RAPD markers (OPE-01, OPE-02, OPE-04, OPE-06, OPE-07, OPE-09, OPE-12, OPE-14, OPE-16, OPE-19, OPE-20, OPE-21, OPE-24 and OPE-25) were best among the 25 markers screened. The RAPD markers were able to distinguish groups among the banana cultivars in eight different clusters among the selected genotypes namely Cluster I- Malbhog, Martaman, Kalibhog and Sobri; Cluster II- Alapan, Poovan

and Champa; Cluster III- Amritpani, Bamandeshi; Cluster IV- Dudhsagar, Rasthali; Cluster V- Krishna Vazai, Manohar; Cluster VI- Chang Monua; Cluster VII- Kanai Bansi and Cluster VIII- Nendran.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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