Original Research Article

Molecular Characterization of Some Indigenous Plantain and Cooking Type Banana Germplasm

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A B S T R A C T

Globally, banana is the maximum distributed fruit crop and is the fourth most important commodity and is grown in more than 130 countries across the world, continuously exhibiting a spectacular growth pattern worldwide in an area of 6507.7 million ha producing 98946 million tones. India is the largest producer of banana in the world. There are diverse germplasms of banana traditionally cultivated in different regions of India having remarkable genetic differences. Traditional procedure to characterize banana plants by morphological descriptors has many limitations. Different communities refer to the local cultivars by different names and lack of clear clone identity in the crop has resulted 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. Randomly Amplified Polymorphic DNA (RAPD) markers are usually preferred for the initiation of this kind of work as the technique is simple, versatile, relatively inexpensive and able to detect minute differences. Progress in the breeding of plantain and banana has been restricted by the complex genetic structure and behavior of cultivated polyploidy *Musa*. Genetic improvement has been hindered due to the large amount of space required for growth and maintenance of plant populations, in addition to the long growth cycle and the low levels of fertility and seed viability characteristic of cultivated genotypes. Molecular marker assisted breeding has the potential to dramatically enhance the pace and efficiency of genetic improvement in *Musa*. A proper classification of *Musa* clones and cultivars is important to assisting the selection of characters for banana breeding. In the present investigation, a detailed study was performed to evaluate the characterization of banana genotypes done by genetical assessment through RAPD markers. For this study, fifteen genotypes were used. By analyzing the molecular characters, we found several clusters and for the conformation of their genetical stability. Evaluating molecular characters of clusters we observed genetical assistance proved that eleven cultivars were fully characterize and they prevailed in same group (ABB group) except Manjeri Nendran and Nendran (AAB).

Introduction

Banana and plantain (Genus: Musa; Family: Musaceae) are fruit crops of great socio-economic significance as they serve as staple food for many millions of people across the tropics and sub tropics. Globally, bananas are grown in more than 130 countries across the world on an area of 8.25 M ha with an annual production of over 100 million tonnes, of which about one third are produced in the African, Asia-Pacific and Latin American and Caribbean regions. Modern cultivated bananas evolved from intra and inter specific hybridization between two wild diploid species, *Musa acuminata* Colla. and *Musa balbisiana* Colla., that contributed the A and B genomes respectively (Simmonds, 1995). Commercially cultivated bananas are seed sterile diploid, triploid or tetraploid clones which are classified into different genome groups as AA, BB, AB, AAA, AAB, ABB, AAAA, AAAB, AABB and ABBB based on a system created by Simmonds and Shepherd (1955). However, many of the cultivars cannot be classified into their respective genome groups, as they show a bias towards either A or B phenotype. Moreover, most of the cultivars cannot be easily distinguished on the basis of their morphology, especially if they are closely related. Practice of assigning local names to cultivars based on fruit and plant characteristics resulting in numerous synonyms and homonyms has created an added confusion in the identification and classification of banana cultivars.

India is recognized as one of the major centers of origin and diversity especially for *M. balbisiana* (Simmonds, 1962). Diversity among commercial cultivars in India is also vast owing to diverse climatic conditions from dry arid zones to humid tropical and cold temperate zones. South Indian states, especially Kerala, Tamil Nadu and Karnataka are endowed with numerous delicate edible diploid *M. acuminata's* also (Uma and Sathiamoorthy, 2002). Analysis of genetic diversity and characterization are central to the effective management of genetic variation. In order to make the best utilization of genetic potential of genotypes for improvement of traits and for adaptation to various stress conditions, genetic study is crucial (Salem *et al.,* 2008). A good understanding of the current status of genetic diversity and adaptive potential of population is a pre-requisite for the successful management of conservation programs. Information on the genetic diversity and phylogenetic relationships within banana germplasm is scarce (Mukunthakumar *et al.,* 2013). With this background, present study attempted to assess the genetic diversity and structure of *Musa* cultivars using molecular markers. Applying the appropriate degree of caution, random amplified polymorphic DNA markers (Williams *et al.,* 1990) can provide invaluable tools to study patterns of genetic variability due to their advantages over other molecular methods, such as less complex and labour-intensive procedures and more arbitrary sampling of the genome.

Lougheed *et al.,* (2000) suggested that concerns regarding the relatively lower resolving power of random amplified polymorphic DNA (RAPD) markers can be ameliorated by the relative ease with which one can increase the number of assayed loci. The use of RAPD markers to genetically fingerprint plants which are morphologically similar or indistinguishable has been established as reliable, efficient and informative as they tend to reside in regions with many repeated sequences, and therefore in non-coding regions, which are more susceptible to mutations, consequently reveal more polymorphisms (Irwin *et al.,* 1998). Thus, they allow better analysis of genetic identity and variation amplifying different regions of the genome and are effective to reveal inter-intra species DNA variability

(Zhao *et al.,* 2015). Random amplified polymorphic DNA markers have been widely used for the identification of genetic relationships, among cultivars, among wild forms and between wild forms and cultivars (Bukhari *et al.,* 2015). Reports are also available supporting that RAPD markers are as effective as AFLP markers for identifying clonal diversity (Albert *et al.,* 2003; Kjolner *et al.,* 2004). In this study, an effort has been done to identify the level of diversity and population structure present in the cultivars distributed in ABB genome groups of *Musa* employing RAPD markers.

A proper classification of *Musa* clones and cultivars is important in assisting the selection of characters for banana breeding. This study therefore describes the use of RAPD markers to evaluate the genetic diversity and relationships amongst different banana cultivars.

Materials and Methods

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

Genomic DNA was extracted from the soft tender leaves of the plant, using standard protocol followed by Varadarajan and Prakash (1991). 100 mg leaf sample was taken and crushed in pre-chilled pestle mortar in liquid nitrogen. The DNA pellet was re-suspended in 100 µl of TE buffer (Tris-HCl 10mM and EDTA 1mM pH 8.0). The quality and quantity of extraction was verified by running each extracted DNA samples in 1% agarose gels stained with ethidium bromide. Samples were kept in -20°C.

RAPD detects polymorphism at random. Here short single (~10 nt) primer was used for PCR amplification. RAPD being a dominant marker produces large number of polymorphic bands so it can differentiate between very closely related organisms too. PCR amplification was performed using the protocol outlined by Williams *et al.,* (1990) with slight modifications. Altogether 10 random decamer oligonucleotide primers were started for screening (Table 2). But only 5 primers showed polymorphism. Ingredients of each reaction included template DNA 25-30 ng, 100 mM each dNTPs, Taq DNA polymerase 0.5 units, $MgCl_2$ 600 mM, 10x PCR reaction buffer and 15 ng of each decamer primer in a total volume of 25 µl.

Amplification was performed in a thermo cycler. Complete reaction consisted of 36 cycles, each cycle consisting of three steps; denaturation at 92°C for 30 seconds, annealing at 38°C for 30 seconds, extension at 72°C for 1 minute, with an initial denaturation at 94°C for 2 minutes and final extension at 72°C for 7 minutes, followed by cooling at 4°C temperature. 15 µl of amplified PCR products were loaded on 1% agarose gel containing ethidium bromide (0.5 µg ml-1 of agarose) at 100 Volt for five hours in TAE buffer (Tris 1.6M, Acetic acid 0.8M, EDTA 40 mM). The gel was photographed and analyzed. A 100 bp ladder was included in each gel as a molecular weight standard.

The bands of 15 genotypes were calculated for 5 different primers, named as OPE 02, OPE 12, OPE 16, OPE 19 and OPE 24. Those bands were calculated in '0' '1' matrix *i.e.,* 0 for absence of band and 1 for presence of band. Then the MI, PIC and Rp were calculated.

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 dendogram was generated using unweighted pair group arithmetic mean method (UPMEGA) using NTSYS pc version 2.1 software for classifying under several clusters (Rohlf, 2000). For Primer characteristics, Polymorphic Information Content (PIC), Marker Index (MI) and Resolving Power (Rp) value was calculated using following formula:

PIC= $2*fi$ (1-fi) where fi= frequency of ith allele

MI= PIC*Average no. of Band*Proportion of polymorphic band

Rp= $\sum I_b$ Where, I_b= 1- $\left|2*(0.5\text{-}p)\right|$, P= proportion of any band

Results and Discussion

Study of genetic diversity assesses the variability exiting within the population. Genetic diversity plays an important role in crop improvement program. However, as banana mainly propagated through suckers and hybridization is rarely possible in this climate the objective of present study was to identify closely or distantly related groups of genotypes. In the presence study statistical method was used for assessing the underline genetic diversity within the population of 15 genotypes of banana collected from different district of west Bengal and other parts of India (Table 1).

Initially three different parameters namely, Taq DNA polymerase concentration, $MgCl_2$

concentration and primer concentration were examined with fixed amount of *Musa* spp. Optimum concentration selected based on clear banding pattern. However, a combination of 1.0 µl of primer and 0.25 µl of Taq DNA polymerase gave best results with addition of 1.0 µl $MgCl_2$ (2.5 mM) in the reaction mixture improve the banding pattern.

In a separate set of experiments, a regime of six different annealing temperatures (25°C, 27°C, 30°C, 35°C, 37°C, 40°C) were tested with 10 standardized concentration primers) and Taq DNA polymerase with cultivars *of Musa* spp. A clear and prominent banding pattern was recorded at these three different annealing temperatures (30°C, 35°C and 37°C). Only five primer gave polymorphic bands. But other did not show any bands (Table 3).

Altogether 10 random decamer oligonucleotide primers (Imperial Life Science Pvt. Ltd. Gurgaon, Haryana, India) were started screening. But only 5 primers showed polymorphism (Table 4). In the beginning of our experiment, 10 decamer primers were screened to study robustness of amplification, reproducibility and score of banding patterns. As a result, from 10 primers, 5 did not produce any polymorphic bands and rest of five primers were used to identify the genotypes to study and those showing polymorphic bands which are suitable for study purpose (Fig. 1).

From the clustering pattern of the genotypes, it revealed that the 15 genotypes were grouped into seven clusters. Due to similarity germplasms like NRCB-7, Baish Chhara and Cuba came under in the same cluster *i.e.* Cluster-I. Behula, Saba and Pantha Bontha Bothisa formed another cluster *i.e.,* Cluster-II. Booditha Bontha Bothisha, Pantharaj, Purulia Collection, Kothia, Green Bombai confied Cluster-III.

Table.1 Banana germplasms collected from various states

Sl. No.	Sample Name	Source
1	Manjeri Nendran	Kerala
2	Nendran	Kerala
3	NRCB- 8	Trichi, Tamil Nadu
4	NRCB- 7	Trichi, Tamil Nadu
5	Baish Chhara (BCB 2)	Hort. Res. & Dev. Farm, Chinsurah, WB
6	Behula	Hort. Res. & Dev. Farm, Chinsurah, WB
7	CUBA 03	IIHR, Banglore, Karnataka
8	Saba	IIHR, Banglore, Karnataka
9	Pantha Bontha Bothisa	Odisha, Bhubaneswar
10	Booditha Bontha Bothisa	Odisha, Bhubaneswar
11	Purulia Collection	Chelyama, Dist- Purulia, WB
12	Pantharaj	Hort. Res. Farm, Kalyani, Nadia, WB
13	Kothia	Bihar
14	Green Bombai	Hort. Res. Farm, Kalyani, Nadia, WB
15	Bloggue	IIHR, Banglore, Karnataka

Table.2 Primers and their sequence used during the study

Sl. No.	Code	Primer sequence (5'-3')
1	OPE 02	GAGACATGCC
2	OPE 05	TTATCGCCCC
3	OPE 08	ACGGCGTATG
4	OPE 10	CCAAGCTTCC
5	OPE 11	CCAGTACTCC
6	OPE 12	TGAGCGGACA
7	OPE 16	AATCGGGCTG
8	OPE 18	GGTCCCTGAC
9	OPE 19	GTGACGTAGG
10	OPE 24	TTCCGAACCC

Table.3 Primers used in the present study and their annealing temperature

Serial no.	Primer name	Primer Sequence (5'-3')	Annealing Temp.
1	OPE 02	GAGACATGCC	37°C
2	OPE 12	TGAGCGGACA	35° C
3	OPE 16	AATCGGGCTG	35° C
4	OPE 19	GTGACGTAGG	37° C
5	OPE 24	TTCCGAACCC	30° C

**Table.4** Primers showed Polymorphism during the present study

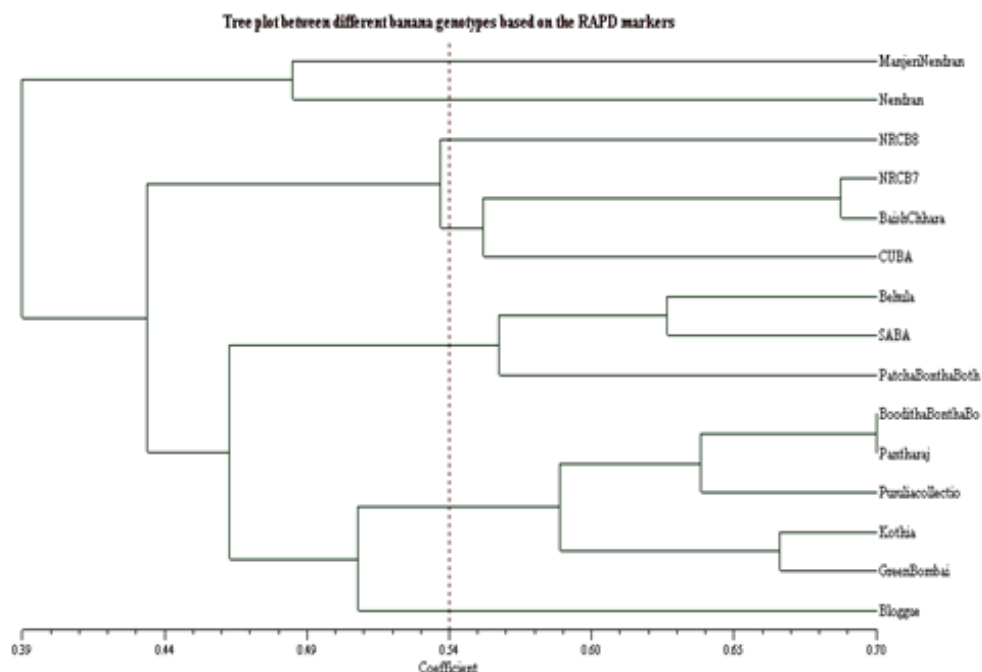| Primer	Total no. of Bands	Polymorphic Bands	% of polymorphism
OPE 02	15	12	80.0
OPE 05	0	0	0
OPE 08	0	0	0
OPE 10	0	0	0
OPE 11	0	0	0
OPE 12	14	14	100.0
OPE 16	15	15	100.0
OPE 18	0	0	0
OPE 19	11	9	81.0
OPE 24	13	11	84.6

Table.5 Five primers showed polymorphism during the study and their characters

Primer	Total no. of Bands	Polymorphic Bands	% of polymorphism	PIC Value	MI Value	Rp Value
OPE 02	15	12	80.0	0.34	2.36	0.52
OPE 12	14	14	100.0	0.32	2.52	0.46
OPE 16	15	15	100.0	0.41	2.82	0.66
OPE 19	11	9	81.0	0.67	4.32	0.39
OPE 24	13	11	84.6	0.30	1.22	0.44

Fig.1 Five primers (OPE 2, 12, 16, 19, 24) showed polymorphic bands

OPE 02

OPE 12

OPE 16

OPE 19

OPE 2
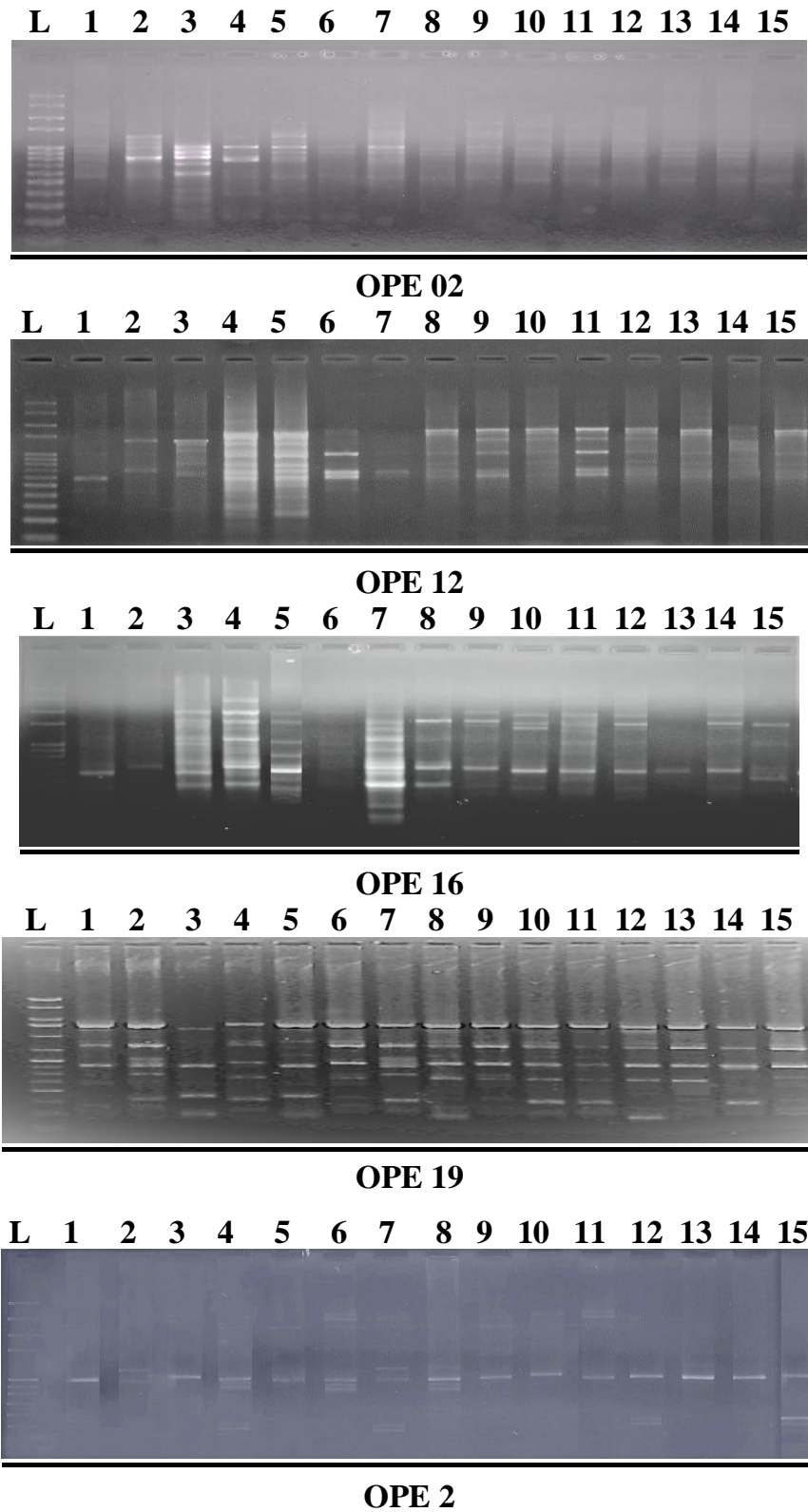
Fig.2 Dendogram showed genetic relationships among 15 banana genotypes under study

Tree plot between different banana genotypes based on the RAPD markers

Whereas Manjeri Nendran, Nendran, NRCB-8 and Bluggoe formed single Cluster differently, those were Cluster IV, Cluster V, Cluster VI, and Cluster VII (Fig. 2) the dendrogram generated using unweighted pair group arithmetic mean method (UPGMA) using NTSYS-pc version 2.1 software resulted in two major clusters.

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 (81.33%) 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). This numerical value also suggests that the species genetic diversity was high and hence enables it to adapt environmental variations.

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 Booditha Bontha Bothisha and Pantharaj isolates, ranging from 70%. NRCB-7 and Baish Chhara 69% similar whereas, Kothia and Green Bombai depicted high level of similarity *i.e.,* 67%. Behula and Saba provided 62.5% similarity which may be a potential of such a high degree of similarity. There was 59% similarity between the two sub clusters (Boodhita Bontha Bothisha, Pantharaj and Purulia Collection. Out of these two *i.e.,* Pantharaj and Purulia Collection were familiar in West Bengal especially Pantharaj from Kalyani, Purulia Collection was originated from Purulia and Budhita Bontha Botisha originated from Orissa, Bhubneswar). Kothia collected from Bihar and Green Bombai originated from Kalyani, West Bengal (Fig. 2). The lowest degree of similarity was shown in between Bloggue and Nendran (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 Bloggue were collected from Karntaka). 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* (19.7-49.1%) (Um *et al.,* 2001), *Poa trivialis* (7-74%) (Rajasekar *et al.,* 2006), *Rhododendron* spp. (26.2-90.6%) (Lanying *et al.,* 2008), *Lathyrus sativus* (13-66%) (Sedehi *et al.,* 2008), Common bean (19-91%) (Tiwari *et al.,* 2005), *Ensete ventricosum* (16-85%) (Birmeta *et al.,* 2002).

Similar studies have also been conducted by others. Bhat and Jarret (1995) found genetic diversity in 57 accessions of Indian *Musa* by RAPD. They randomly screened 60 primers, out of them 49 gave reproducible amplification product of amplified DNA. Bands per amplification differed from 1 to 24. Uma *et al.,* (2006) using RAPD marker, studied interspecific relationship of wild *Musa balbisiana* Colla by RAPD markers. 16 types of wild banana were collected from different regions of India. 80 primers were used and 34 primers produced reproducible bands. Among them, 4 primer gave more than 5 reproducible bands. In another research 21 commercially produced banana cultivars from South India were examined through RAPD analysis (Venkatachalam *et al.,* 2008). Where 50 primers out of 75 primers produced robust amplification products.

Saha Roy *et al.,* (2010) also found that 72.7% polymorphism in between *Musa* spp. which reveal two major subgroup of banana *i.e.,* from plains and hills. 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 Booditha

Bontha Bothisha and Pantharaj isolates were same genotypes as revealed by their maximum similarity coefficient. Essentially little morphological variation between then 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 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).

OPE 24 has lower PIC value, MI value, and also lower Rp value (Table 5). So this primer is least preferable. OPE 19 has lowest Rp value (0.39) but having highest MI value *i.e.,* 4.32 and highest PI value *i.e.,* 0.67. OPE 16 has shown 100% polymorphism. It has a good MI value (2.82) and PIC value (0.41). Moreover, OPE16 has the highest Rp value *i.e.,* 0.66. Like OPE 16, OPE 12 has also shown 100% polymorphism, having good MI (2.52), PIC (0.32) and Rp value (0.46). OPE 02 has shown 2.36 MI value, 0.34 PIC value, and 0.52 Rp value. Based on PIC, MI and Rp value it can be concluded that OPE 16 and OPE 19 are the best primer for genetic diversity study of *Musa*.

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