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*Full Length Research Paper*

# Evaluation of genetic diversity between 27 banana cultivars (*Musa* spp.) in Mauritius using RAPD markers

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**Cultivated bananas (*Musa* spp.) are mostly diploid or triploid cultivars with various combinations of the A and B genomes inherited from their diploid ancestors *Musa acuminata* Colla. and *Musa balbisiana* Colla. respectively. Random amplified polymorphic DNA (RAPD) markers were used to establish the relatedness of 27 accessions in the Mauritian *Musa* germplasm. 15 decamer primers produced a total of 115 reproducible amplification products, of which 96 were polymorphic. Computation of the genetic distances shows that similarities ranged from 0.3 to 1.0 with an average of 0.51. With a few exceptions, cluster analysis differentiated pure A containing cultivars from those containing at least one B genome. This paper answers long standing questions on the taxonomic placement of the cultivar 'Banane Rouge' by providing the basis for its classification within the homogenomic A cultivars. The results presented here also contribute to narrowing the gaps in our current understanding of the migration path of bananas and the emergence of secondary centers of diversity.**

**Key words:** Dendrogram, genetic distances, genetic diversity.

## Introduction

Bananas (*Musa* spp.) are amongst the most important food crops in the world. Global banana production has been estimated to be about 99 million tonnes annually, mostly produced by tropical countries (FAO, 2003). Despite the importance of bananas in trade and commerce, little is known of the genetics of its agronomically important traits (Loh et al., 2000). The widespread threat to banana cultivation by several pathogens in recent years has brought a renewed interest in *Musa* breeding programs. Many improved varieties released from such programs 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 programs (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).

Cultivated bananas are referred to by their genome groupings. The crop encompasses a range of diploids, triploids and tetraploids. 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. According to this system, cultivated dessert and East-African highland bananas are classified as AAA whilst plantains are AAB. There exist other genome combinations, for example ABB and ABBB. They occur naturally or are produced by artificial hybridization (Stover and Simmonds, 1987).

The taxonomy of cultivated bananas has long been a contentious issue and because it relies heavily on morphology, the literature is plagued with contradictions.

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For instance, based on molecular data, Pillay et al. (2000) have shown that the clones 'Monthan Saba' and 'Bluggoe', previously classified as BBB on the basis of morphological characteristics belong to the ABB group. Similarly, the clone 'Klue Tiparot', originally regarded as a tetraploid ABBB has been reclassified as a triploid ABB (Jenny et al., 1997; Horry et al., 1998). In addition to the subjective nature of classification schemes based on morphology, the frequent occurrence of somaclonal variation during *in vitro* propagation and the close genetic relationship between cultivars are major hurdles for the correct identification of clones based on morphological descriptors only (Jarret and Gawel, 1995).

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. As a more reliable alternative, various DNA fingerprinting techniques have been used to study the genetic diversity and taxonomy of cultivated bananas. These include isozyme analysis (Bhat et al., 1992), restriction fragment length polymorphism (RFLP) (Jarret et al., 1992; Kaemmer et al., 1992; Bhat et al., 1994), 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).

Random amplified polymorphic DNA (RAPD) (Williams et al., 1990; Welsh and McClelland, 1990; Howell et al., 1994; Pillay et al., 2000;) is another such technique which allows one to easily back taxon names with a molecular identification system in the form of a barcode inherent to the plant. Using this technique, an unlimited number of polymorphic bands can be produced with relative ease from minute amounts of genomic DNA (Welsh and McClelland, 1990) allowing simultaneous screening of a large number of accessions. 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).

The island of Mauritius hosts several different banana cultivars. However, few studies have been conducted to establish the genetic relationship between these cultivars. In a survey of the Musaceae on the island, Pitot (1905) recorded the first introduction of a *Musa* clone (cv. 'Banane graine') from Bantam in 1606. This cultivar had also been referred to as 'Pisang cato' (Céré, 1789), *Musa sapientum* Linn. and *Musa seminifera* Lour. (Rouillard and Guého 2002). The cultivar 'Banane Rouge', which has been variously referred to as *Musa paradisiaca* (Thompson, 1816) or *Musa rosea* (Bojer, 1837) was probably introduced at the same period. However, due to incomplete records, there is little information on its origin and relationship with cultivated bananas. Similarly, the cultivar 'Dwarf Cavendish' (*Musa* AAA), introduced in Mauritius in 1826 from southern China (Stover and Simmonds, 1987), has been variously known as *M. paradisiaca* humilis (Bojer, 1837) or *M. chinensis* (Cantley, 1880) before its final classification as 'Dwarf Cavendish'. In a more recent study, Rouillard and Guého (2002) recorded the existence of a peculiar series of cultivars, highly appreciated for its taste and known for years by the Mauritian population as 'Gingeli'. These cultivars have been grown almost exclusively in a back-yard cultivation system and thus, their origins and genetic relationship with cultivated bananas remains shrouded in mystery.

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

## MATERIALS AND METHODS

### Plant material

A total of 27 accessions were sampled for the present study (Table 1). These plants were chosen to represent a wide range of genomic groups. Specimens were collected from the *Musa* germplasm of the Agricultural Research and Extension Unit (AREU), Richelieu, Mauritius.

### DNA extraction

Prior to DNA extraction, the leaves were surface sterilized using the procedure described by Zhang et al. (1997). Total genomic DNA was extracted using the protocol of Gawel and Jarret (1991). Integrity of the DNA was evaluated by electrophoresis on 1.5% agarose gel and concentration measured using a GeneQuant pro RNA/DNA calculator (Amersham Pharmacia, UK).

### RAPD reactions

Optimized reaction mixtures for RAPD analyses consisted of 50 ng template DNA, 3.5 mM $MgCl_2$, 200 µM dNTPs, 0.5 U HotStar© Taq DNA polymerase (Qiagen, Germany), 2.5 µM primer (Operon Technologies, USA), 1 X PCR buffer, 250 ng BSA and 1 X Q solution for a total reaction volume of 25 µL. Amplifications were carried in a Dyad© DNA Engine PTC-220 (MJ Research, USA) with the following cycling parameters: an initial denaturation at 94°C for 15 min, 3 cycles of 25 sec at 94°C, 25 sec at 35°C and 2 min at 72°C followed by 40 cycles of 25 sec at 94°C, 25 sec at 37°C and 2 min at 72°C. After a final extension step at 72°C for 7 min, reactions were ended with an indefinite hold at 4°C. PCR products were resolved by electrophoresis alongside 2 molecular weight markers; DNA ladder II (m1) (GeneChoice , USA) and 100 bp DNA ladder (m2) (Invitrogen, USA) on 1.5% NuSieve© 3:1 agarose (Cambrex BioScience, USA) gels in 1 X TAE buffer, stained with ethidium bromide and photographed under UV.

### Data analysis

The RAPD band patterns were analysed using the Quantity One©

**Table 1.** List of *Musa* cultivars used in the present study.

| No. | Cultivar Name | Subgroup/Ancestry | Accession Number |
|---|---|---|---|
| **Tetraploid AAAA** | | | |
| 1. | FHIA02 | Williams(AAA) x SH-3393(AA) | uomacc.#d1 |
| **Triploid AAA** | | | |
| 2. | Dwarf Cavendish | Cavendish | areuacc.#n1 |
| 3. | Williams | Cavendish | areuacc.#j8 |
| 4. | Grande Naine | Cavendish | areuacc.#h10 |
| 5. | Yangambi Km5 | - | uomacc.#f6 |
| **Diploid AA** | | | |
| 6. | Pisang Mas | Sucrier | areuacc.#i10 |
| 7. | Lidi | - | areuacc.#l7 |
| 8. | Rose | - | uomacc.#b4 |
| **Tetraploid AAAB** | | | |
| 9. | FHIA21 | French Plantain(AAB) x SH-3142(AA) | areuacc.#a7 |
| 10. | FHIA18 | Prata Enane(AAB) x SH-3142(AA) | uomacc.#d2 |
| 11. | SH-3640 | Dwarf Prata(AAB) x SH-3393(AA) | areuacc.#h7 |
| **Triploid AAB** | | | |
| 12. | Pisang Ceylan / Mysore | Mysore | areuacc.#l11 |
| **Triploid ABB** | | | |
| 13. | Klue Tiparot | - | uomacc.#b5 |
| **Diploid AB** | | | |
| 14. | Kisukari | - | areuacc.#i7 |
| 15. | Ney Poovan | - | uomacc.#b1 |
| **Unknown Genotypes** | | | |
| 16. | Gingeli BES | - | areuacc.#e6 |
| 17. | Gingeli Puchooa | - | areuacc.#e3 |
| 18. | Gingeli Modely | - | areuacc.#d6 |
| 19. | Gingeli Philibert | - | areuacc.#c4 |
| 20. | Banane Rouge | - | areuacc.#h1 |
| 21. | Mammoul | - | areuacc.#g4 |
| 22. | Gingeli Maroussem | - | areuacc.#c3 |
| 23. | Gingeli FUEL | - | areuacc.#d3 |
| 24. | Gingeli Savannah | - | areuacc.#f4 |
| 25. | Ollier | - | areuacc.#l1 |
| 26. | Banane Carrée | - | areuacc.#un |
| 27. | Seeded Banana | - | uomacc.#un |

1D analysis software (Biorad, USA). The dendrogram showing relationships between the different cultivars was generated using Nei and Li's Dice coefficient with UPGMA clustering (Figure 2). The tree thus obtained was subjected to bootstrapping analyses with 1000 replicates to determine the robustness of each node.

## RESULTS

### RAPD reaction profiles

A total of 15 decamers from primer kits OPC, OPAJ, OPAK and OPB were used to investigate the variability amongst the different *Musa* accessions. Typical results obtained are shown in Figure 1 for primer OPB-07 and OPB-17. The RAPD reactions generated 115 easily scored amplification products ranging in size from 150 to 2600 bp, of which 96 were polymorphic, representing an average of 6.9 polymorphic bands per primer. The level of polymorphism detected was 83%, which agrees fairly well with previous studies (Visser, 2000; Crouch et al., 1999).

### Genetic relationships

A pairwise comparison of the 27 accessions shows that the similarity coefficient ranged from 0.3 between the cul-

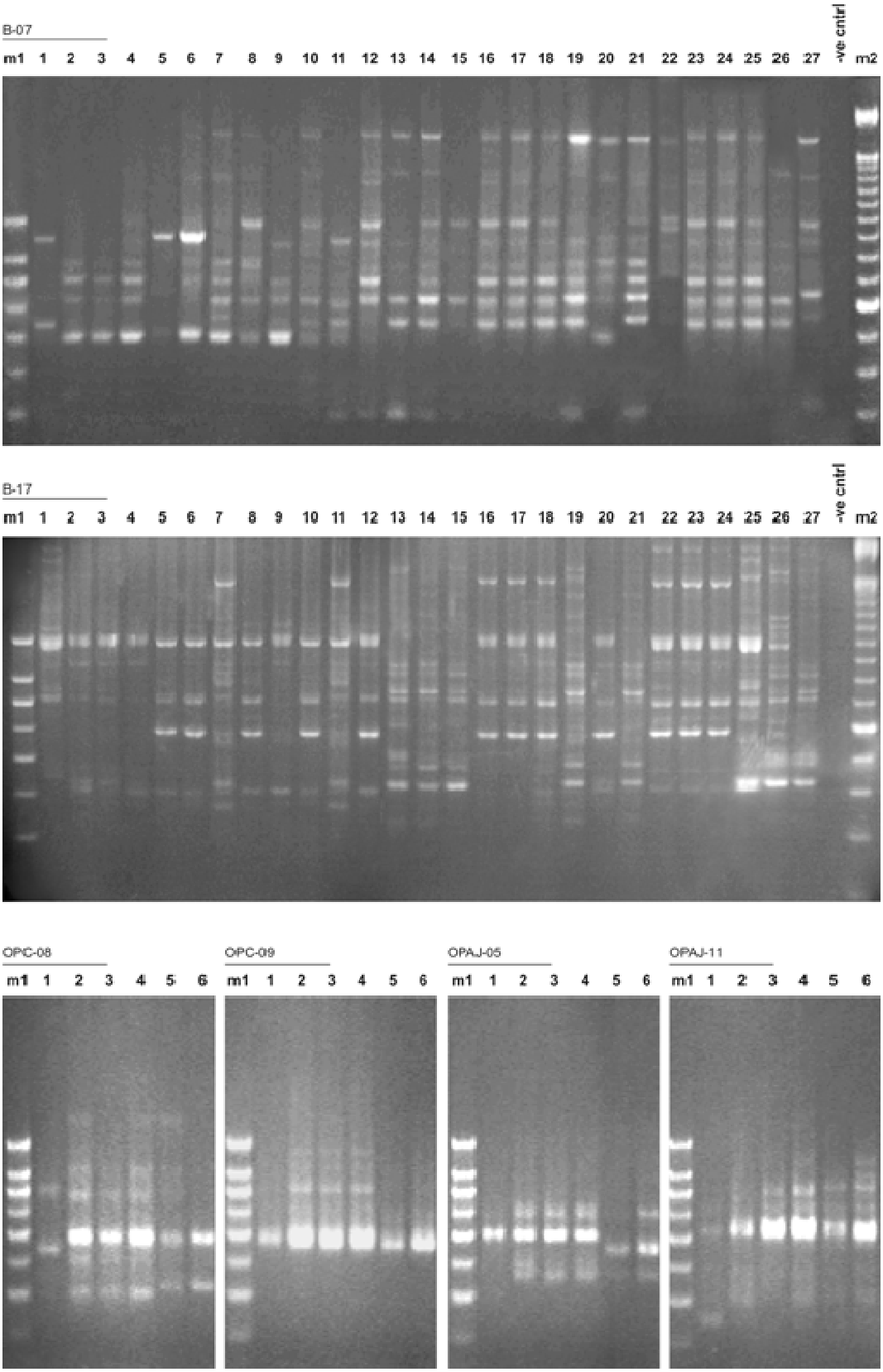


**Figure 1.** Amplification profiles of the 27 *Musa* cultivars. The lanes are labelled as in Table 1. A negative control without template DNA is included and labelled –ve cntrl. The code of each primer is indicated above their respective profile.

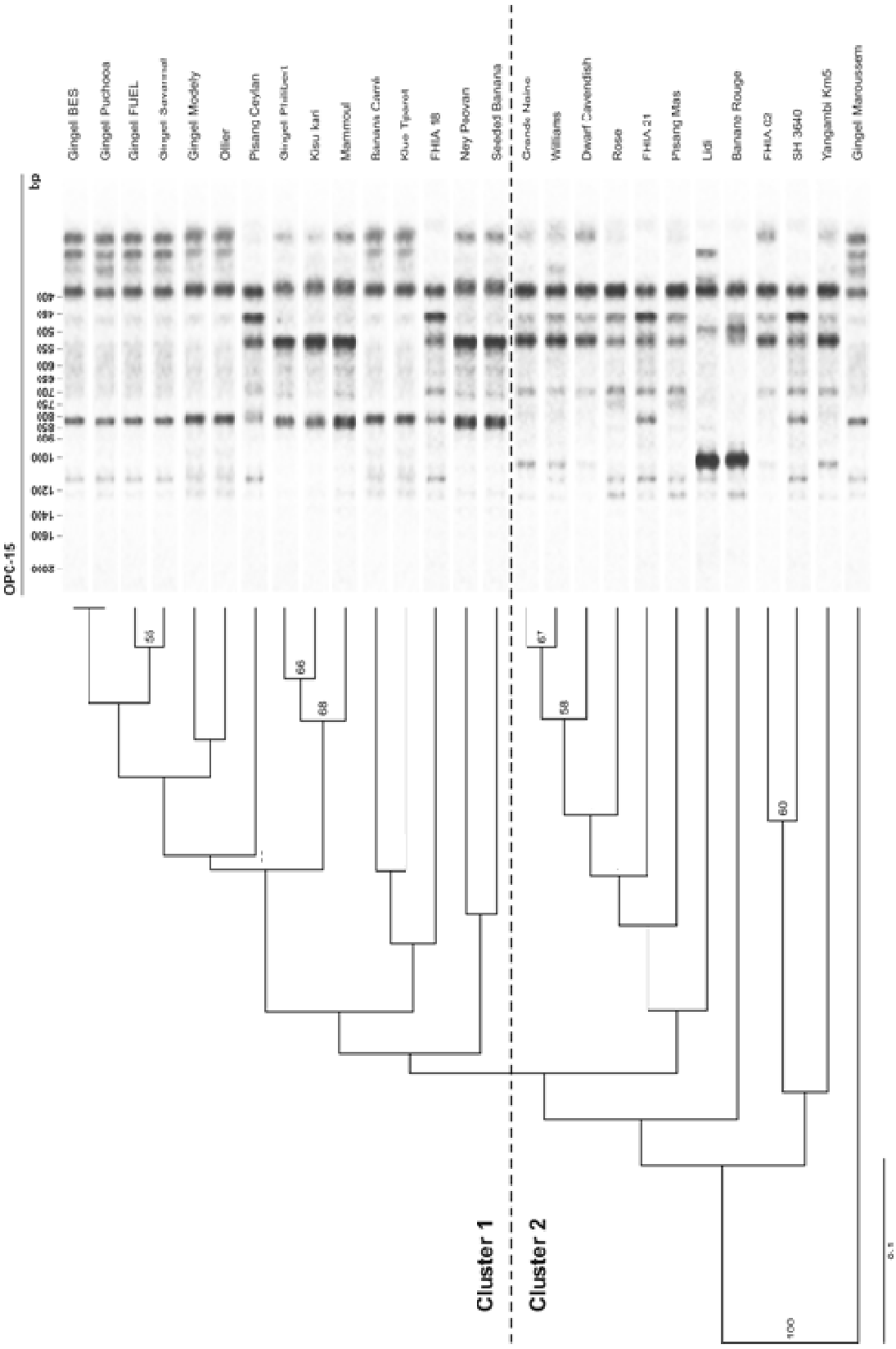


**Figure 2.** Dendrogram showing the relationship between the 27 *Musa* accessions based on Nei and Li's Dice coefficient along with the amplification profile obtained with primer OPC-15. The main clusters are indicated and bootstrap values >50% are displayed on the branches.

tivars ‘Pisang Ceylan’ and ‘FHIA02’ to 1.0 between ‘Gingeli BES’ and ‘Gingeli Puchooa’. The dendrogram generated from this data shows two main clusters with Gingeli Maroussem as outgroup (see Figure 2). Cluster 1 consists of four main subclusters and the first one comprises of the local cultivars ‘Gingeli BES’, ‘Gingeli Puchooa’, ‘Gingeli Fuel’, ‘Gingeli Savannah’, ‘Gingeli Modelly’, ‘Ollier’ as well as the imported variety ‘Pisang Ceylan’ (AAB). The second subcluster encompases Kisu Kari (AB) and the two local cultivars ‘Gingeli Philibert’ and ‘Mammoul’ while the third subcluster includes ‘FHIA 18’ (AAAB), ‘Klue Tiparot’ (ABB) and the local cultivar Banane carrée. The last subcluster consists of Ney Poovan (AB) and the Seeded Banana.

On the other hand, cluster 2 mostly groups the homogenomic A cultivars with ‘Grande Naine’ (AAA), ‘Williams’ (AAA), ‘Dwarf Cavendish’ (AAA), ‘Rose’ (AA), FHIA21 (AAAB), ‘Pisang Mas’ (AA) and ‘Lidi’ (AA) forming the main subcluster while FHIA02 (AAAA), SH3640 (AAAB) and Yangambi km5 (AAA) make up the second subcluster. The local cultivar ‘Banane Rouge’ is also a member of cluster 2.

## DISCUSSION

Analysis of the genetic relationship between the 27 cultivars based on RAPD markers revealed that, with the exception of ‘FHIA21’ (AAAB) and ‘SH-3640’ (AAAB), the putative homogenomic A cultivars were distinctly separated from those containing at least one B genome (Figure 2). This is in accordance with the work of Pillay et al. (2000) who have been able to differentiate pure A containing cultivars from those with at least one B genome in *Musa*. The inclusion of the B genome containing cultivar ‘SH-3640’ within cluster 2 and its close grouping with ‘FHIA02’ is however explained by the fact that it shares a common ancestry with the latter cultivar. Both of them have as parental stock, the cultivar ‘SH-3393’.

Whilst our result cannot conclusively resolve diploid AA from triploid AAA clones, it does indicate that most cultivars included within cluster 2 are homogenomic A cultivars. This implies that ‘Banane Rouge’ is effectively a pure A cultivar. Thompson (1816) and later Bojer (1837) referred to the cultivar ‘Banane Rouge’ as *M. paradisiaca* and *M. rosea* respectively. Both botanical names were introduced to describe AAB cultivars. Due to the lack of complete records, no studies have unequivocally shown that ‘Banane Rouge’ belongs to this genome group. The data presented here, on the other hand, supports the close clustering of ‘Banane Rouge’ with the diploid AA cultivars ‘Lidi’ and ‘Pisang Mas’, both of which originate from the Indo-Malaysian area.

Rouillard and Guého (2002) highlighted the similarities between the ‘Gingeli’ cultivars and the cultivars ‘Sucrier’ (AA) and ‘Figue Sucrée’ (syn. ‘Pisang Mas’). However, neither description is in agreement with our results. The separation of most of the ‘Gingeli’ cultivars from the major ‘Cavendish’ subtypes in Figure 2 and the inclusion of the putative B containing cultivars ‘FHIA18’ (AAAB) and ‘Ney Poovan’ (AB) within cluster 1 suggest that they possess at least one B genome. From the available literature, it is not possible to trace back the introduction of the ‘Gingeli’ cultivars to Mauritius. Nain and Jaufeerally-Fakim (2002) suggested that the salient morphological characters of these cultivars would tend to liken some of them to the ‘Silk’ (AAB) variety. This would be consistent with our results on account of their possession of at least one B genome. In support of this, the majority of the ‘Gingeli’ cultivars are found clustered with the cultivar of Malaysian origin ‘Pisang Ceylan’ (AAB). On the other hand, ‘Gingeli Maroussem’ is found in the same cluster as the homogenomic A cultivars, separate from the rest of the ‘Gingeli’ series. The reasons for this relationship need further clarification.

De Langhe et al. (1999) proposed that the bananas of the Indian Ocean formed part of an ‘Indian Ocean Complex’ of cultivars with a strong representation of germplasms from India and Southeast Asia. Our results show that the cultivar ‘Gingeli Philibert’ and ‘Mammoul’ are consistently found clustered with ‘Kisukari’ at a similarity level near 70%. With the cultivar ‘Kisukari’ being a Kenyan accession, this relationship could indicate that Mauritius or the neighbouring islands could have, in a distant past, been a link from the primary centres of origin in Asia to the secondary diversity centres in Africa. However, none of the ‘Gingeli’ cultivars are found clustered with ‘Klue Tiparot’ (ABB) or ‘Ney Poovan’ (AB) which find their roots in Thailand and India respectively. Rather, the cultivar ‘Banane Carrée’ described as an AAB triploid by Rouillard and Guého (2002) is found in this cluster.

In conclusion, the lack of complete records on introduced banana cultivars has confounded efforts to classify them. Previous descriptions of these cultivars were based solely on morphological features leading to nomenclature issues. We showed here that the use of RAPD based fingerprinting is a more appropriate alternative to determine the genetic diversity and relationship between the different cultivars. However, more powerful and reliable markers are required for the accurate identification of *Musa* clones and cultivars. With a higher multiplex ratio, the AFLP technique has proved to be extremely effective in discriminating between closely related *M. acuminata* taxa at the sub-specific level (Wong et al., 2001). It will be worth using this technique to obtain a more precise taxonomic placement for the cultivars presented here.

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