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*Short Communication*

# Analysis of B-genome derived simple sequence repeat (SSR) markers in *Musa* spp.

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**A study was conducted to investigate the genetic variability between 40 *Musa* genotypes maintained at the *Musa* germplasm collection of the International Institute for Tropical Agriculture, Ibadan using nine B-genome derived simple sequence repeat (SSR) markers. The nine primers produced reproducible and discrete fragments and generated a total of 23 alleles with an average of 2.1. The hierarchical cluster analysis showed clusters of diploid cultivars separate from triploid ones (with the exception of TMB149 (BB) and TMB131 (AB)). Average gene diversity was He = 0.412, and differentiation, given by the fixation index ($F_{ST}$) was low at 0.131.**

**Key words:** Banana, genetic diversity, gene differentiation, plantain.

## INTRODUCTION

*Musa* is a genus of giant perennial herbs belonging to the Musaceae family of the order Scitamineae (Simmonds, 1995). Edible bananas originated from two wild diploid species, *M. acuminata* (A- genome) and *M. balbisiana* (B-genome), and have been classified into different groups according to their genome composition (Simmonds and Shepherd, 1955).

Several methods have been used to investigate the genetic variability present in *Musa* germplasm. The development and application of technologies based upon molecular markers provide the only tools that are able to reveal polymorphism at the DNA sequence level, which are adequate to detect genetic variability between individuals and within populations (Kresovich, 1995). Microsatellites or simple sequence repeats (SSRs) are among several molecular markers used to characterize and assess genetic variability of the genus *Musa*, because they are highly polymorphic, multi-allelic, co-dominant, reproducible, easy to interpret, and amplified via polymerase chain reaction (PCR) (Crouch et al., 1999).

The objective of this study was to contribute to the characterization of the genetic resources of *Musa* accessions using B-genome derived Simple Sequence Repeat (SSR) markers.

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## MATERIALS AND METHODS

A total of 40 *Musa* accessions including 23 banana and 17 plantain accessions, obtained from the *Musa* germplasm collection of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were used in the study. Young leaves from each accession were excised from the mother plant and used in this study.

Genomic DNA of each of the plant genotypes was extracted using a modified method of Dellaporta et al. (1983) and quantified by agarose gel electrophoresis. A primer test was performed on 44 *Musa* SSR markers (synthesized by MWG Biotech, Inc) with five *Musa* accessions TMP115, TMP20, TMB153, TMB156 and TMB135, then the polymorphic primers were used to genotype the 40 accessions using the method described by Crouch et al. (1999). PCR products were separated on a 6% polyacrylamide gel and visualized by silver staining.

Genetic similarity among genotypes were evaluated using the unweighted pair grouping with arithmetic average (UPGMA) cluster method of Nei's genetic distances (Sneath and Sokal, 1973). A dendrogram of genetic similarity was generated with the NTSYS-PC computer programme version 2.02 (Rohlf, 1997) . Genetic diversity among the accessions was estimated using the software package GEN-SURVEY (Vekemans and Lefebvre, 1997). For all loci and all accessions, total heterozygosity (Ht) and the proportion of among-accession differentiation (Gst) were estimated according to Nei (1978). Genetic differentiation was quantified by the F-statistics estimator (Fst) as described by Weir and Cockerham (1984) using FSTAT 2.9 (Goudet, 1995).

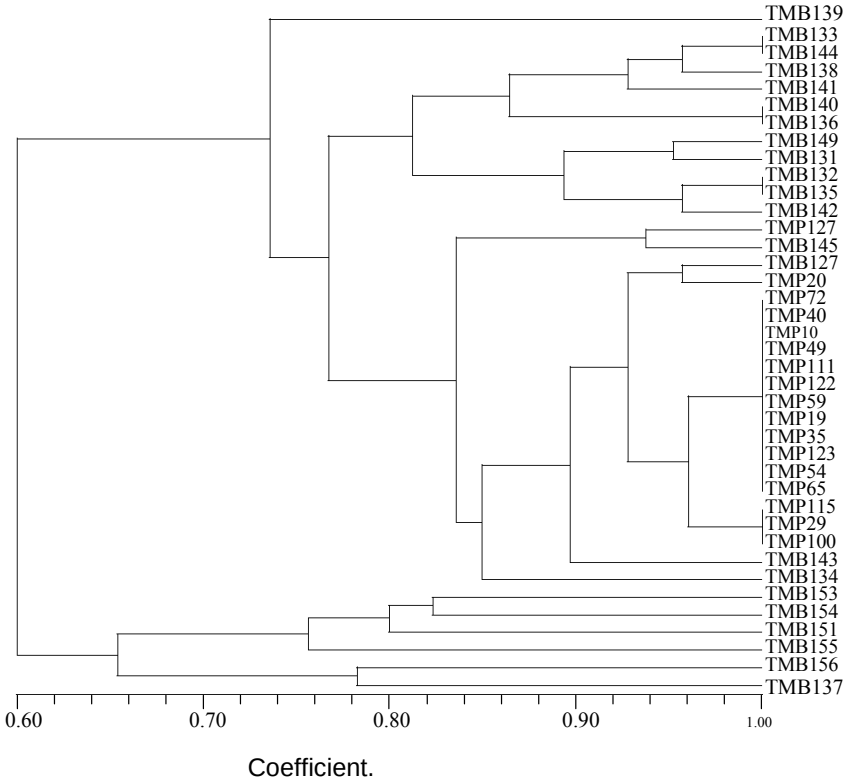


**Figure 1.** Dendrogram showing genetic relationship between *Musa* genotypes analysed with microsatellites.

## RESULTS AND DISCUSSION

Of a total of 44 primers were tested to assess the microsatellite polymorphism in five *Musa* accessions, nine primers (20%) yielded amplification products and were then used to screen all 40 *Musa* accessions. Each primer gave well-defined discrete banding patterns, and a total of 23 alleles were generated and scored as either presence (1) or absence (0) in the accessions. The mean number of alleles per primer was 2.56. Such low number of amplification products from *Musa* SSR's has been reported elsewhere. Creste et al. (2004) used 33 primers to investigate genetic diversity in *Musa* diploid and triploid accessions from Brazillian banana breeding program, and only 15 primers (45.5%) generated amplification products. Since the primers used in this study are derived from the B-genome in *Musa*, it is likely that the primer-anchoring sequences flanking the microsatellite loci might differ sufficiently to restrict amplification of products. Thus, more markers are needed for genetic analysis in *Musa*.

### Genetic similarity and cluster analysis

The dendrogram derived from similarity analysis between the *Musa* accessions is shown in Figure 1. A quick glance at the dendrogram indicates two main cluster groups at 64% similarity. The highest similarity values were observed at 100% similarity among several accessions. All triploid *Musa* accessions were clustered together except TMB143, TMB134 and TMB137. Similarly, diploid accessions were clustered together except TMB149 and TMB131. All plantain accessions were sandwiched between the triploid and diploid banana clusters except the tetraploid plantain, TMP127 (AABB).

### Genetic diversity and differentiation among accessions

High genetic polymorphism for SSR loci was observed among accessions. All the loci were polymorphic among the bananas while 66.7% were polymorphic among the plantains (Table 1). Average gene diversity was He = 0.411 and the average proportion of observed heterozygous individuals was high (Ho = 0.630), which confirms *Musa*'s highly heterozygous nature. F-statistics for the 40 *Musa* accessions are given for each SSR locus as well as their averages over loci (Table 2). The estimates of genetic differentiation within the accessions (Gst = 0.08, Fst = 0.13) are lower than the average reported for crop species (Gst = 0.34) classified for three traits; taxonomic status (dicots vs monocots), life form (annual vs perennial) and mating system (predominantly

**Table 1.** Gene diversity analysis among *Musa* accessions by genome group.

| Population | N | # loc. | # loc_P | P | K | K_P | Ho | He | Hec | Fis |
|---|---|---|---|---|---|---|---|---|---|---|
| Banana | 20 | 9 | 9 | 100 | 2.3 | 2.3 | 0.6164 | 0.4828 | 0.4954 | -0.2282 |
| Plantain | 17 | 9 | 6 | 66.7 | 1.9 | 2.2 | 0.6385 | 0.3408 | 0.3513 | -0.8219 |
| Mean | | | | 83.33 | 2.11 | 2.25 | 0.6274 | 0.4118 | 0.4234 | -0.5251 |
| S.D | | | | 23.57 | 0.31 | 0.12 | 0.0156 | 0.1004 | 0.1018 | 0.4198 |

N = mean no of individual spp,
# loc. = no of loci,
# loc_P = no of polymorphic loci, P
= percentage of polymorphic loci,
K = mean no of alleles per locus,
K_P = mean no of alleles per polymorphic locus,
Ho = mean observed heterozygosity,
He = mean expected heterozygosity (gene diversity),
Hec = mean expected heterozygousity corrected for small sample sizes, and
Fis = weighted average inbreeding coefficient with correction for small sample sizes.

**Table 2.** F-statistics over the 40 *Musa* accessions for each SSR locus.

| Locus | Fit | Fst | Fis |
|---|---|---|---|
| Mb 1-18 | -0.825 | 0.009 | 0.106 |
| Mb 1-52 | -0.184 | 0.151 | -0.395 |
| Mb 1-100 | 0.098 | 0.199 | -0.126 |
| Mb 1-134 | 0.077 | 0.348 | -0.416 |
| Mb 1-139 | -0.773 | 0.019 | -0.808 |
| Mb 1-147 | -0.750 | 0.020 | -0.786 |
| Mb 1-148 | 1.000 | 0.030 | 1.000 |
| Mb 1-149 | -0.090 | 0.192 | -0.349 |
| Mb 1-174 | -0.731 | 0.004 | -0.739 |
| **Overall Loci** | **-0.281** | **0.131** | **-0.474** |

Fit = overall inbreeding coefficient,
Fst = fixation index, and
Fis = average inbreeding coefficient within accessions.

selfing, mixed-mating, or predominantly outcrossing), as compared with wild uncultivated plants and also for the average of outcrossing species (Gst = 0.234, Hamrick and Godt 1997). The overall inbreeding coefficient (Fit = -0.28) indicates no inbreeding overall which is not unusual in *Musa.*

In conclusion, this study shows the genetic diversity within *Musa* germplasm collection with one or more B-genome pedigree. There are several limitations in breeding and genetic improvement in *Musa*, such as the flowering; therefore selecting the most suitable parents that would result in higher diversity is crucial for significant genetic progress.

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