THE JOURNAL OF AGRICULTURE
OF THE UNIVERSITY OF PUERTO RICO

Issued by the Agricultural Experiment Station of the University of Puerto Rico, Mayagüez Campus, for the publication of articles and research notes by staff members or others, dealing with scientific agriculture in Puerto Rico and elsewhere in the Caribbean Basin and Latin America.

VOL. 93 JANUARY AND APRIL 2009 No. 1-2

Ploidy level and genomic composition of *Musa* spp. accessions at the USDA-ARS Tropical Agriculture Research Station[1,2]

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J. Agric. Univ. P.R. 93(1-2):1-21(2009)

ABSTRACT

Plant germplasm collections serve as repositories for important genes. However, insufficient and inaccurate characterization of the genetic diversity in a collection can slow or can prevent full utilization of genetic resources. Bananas and plantains (*Musa* spp., Colla) are some of the most important food crops in the world. Germplasm characterization efforts in *Musa* have focused mostly on agronomic and morphological traits although with the advent of molecular markers genotypic characterization efforts are increasing. Genomic composition in *Musa* is based on a plant's ploidy level and on whether it is of a hybrid origin. Genomic compositions of *Musa* spp. have been associated with disease and insect resistance, production and flavor characteristics. The *Musa* spp. collection of the USDA-ARS Tropical Agriculture Research Station consists of 135 accessions, many of which are of unknown genomic composition. In an effort to better characterize the collection, RAPD and PCR-RFLP markers, as well as flow cytometry, were employed to determine genomic composition and ploidy level. Plant acces-

[1]Manuscript submitted to Editorial Board 7 August 2008.

[2]The authors would like to thank Dr. George Ude and Mr. Scott Hyndman for their critical review of early versions of the manuscript.

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sions maintained in the collection belong to several *Musa* species and their hybrids with different ploidy levels. In addition, several differences in ploidy as well as genomic composition were identified when comparing findings in this study to those reported in the literature.

Key words: *Musa*, ploidy, PCR-RFLP, flow cytometry, characterization

RESUMEN

Nivel de ploidía y composición genómica de accesiones de *Musa* spp. en la colección del USDA-ARS Tropical Agriculture Research Station

Los bancos de germoplasma sirven como fuente de genes importantes. Sin embargo, la falta de caracterización o caracterización inexacta de la diversidad dentro de una colección reduce y puede prevenir el uso máximo de los recursos genéticos. Los guineos y plátanos (*Musa* spp., Colla.) son algunos de los cultivos más importantes como fuente alimenticia en el mundo. La mayoría de los esfuerzos para la caracterización en el género *Musa* se han enfocado especialmente en rasgos agronómicos y fenotípicos, pero con el desarrollo de técnicas moleculares la caracterización genotípica está progresando. La composición genómica en *Musa* se basa en el nivel de ploidía como también en si es de origen híbrido. Las diferentes composiciones genómicas se asocian con ciertas características como son la resistencia a insectos y patógenos, la producción y el sabor. La colección de *Musa* spp. de la Estación de Investigación de Agricultura Tropical del USDA-ARS consiste de 135 accesiones, de las cuales solo a un porcentaje bajo se les conoce la composición genómica exacta. En un esfuerzo por caracterizar mejor la colección se utilizaron las técnicas de RAPDs y PCR-RFLPs al igual que citometría de flujo para determinar la composición genómica. Las plantas de la colección pertenecen a varias especies de *Musa* y a sus híbridos, y tienen ploidía variada. Además, se encontraron diferencias en ploidía así como en composición genómica cuando se compararon los resultados de este estudio con aquellos resultados reportados en la literatura.

Palabras clave: *Musa*, ploidía, PCR-RFLP, citometría de flujo, caracterización

INTRODUCTION

Bananas and plantains (*Musa acuminata* Colla., *Musa balbisiana* Colla., *Musa* hybrids) are an important source of food and fiber in many regions of the world. *Musa* spp. cultivars are currently grown in more than 100 countries throughout the tropical and subtropical regions of the world. For the 2005 calendar year approximately 105 million metric tons were grown worldwide (INIBAP, 2006). According to the Food and Agriculture Organization of the United Nations (FAOSTAT, 2006), total world exports for bananas were over 15.9 million tons in 2006.

Musa species have been severely threatened by genetic erosion caused by poor management of field collections, habitat loss, natural disasters, and insect and disease pests. Presently, many farmers and the banana export industry rely on only a small portion of the known diversity in *Musa*, mainly 'Cavendish' types, which are productive yet extremely susceptible to insect and disease pests.

Musa spp. taxonomy is complex; the genus is comprised of several sections of which the *Musa* section (formerly *Eumusa*) is the most important since it contains most of the cultivated varieties known today. The complexity of the taxonomy is also due to the fact that the plant's ploidy levels vary. Originally, two wild diploid species were described, *Musa acuminata* (designated AA) and *M. balbisiana* (designated BB) (Simmonds, 1962; Simmonds and Shepherd, 1955). Later, it was discovered that these two species could hybridize in nature as well as by conventional plant breeding techniques (Stover and Buddenhagen, 1986). A cross between these two diploid species yields a hybrid AB genome. Further hybridization and polyplodization results in varying ploidy levels including triploids (e.g., AAB) and tetraploids (e.g., AABB). Most cultivated *Musa* spp. fall into one of several genomic composition groups. All true plantains and cooking bananas are considered to have an AAB and ABB genomic composition, respectively, whereas the export banana industry is based on a triploid AAA or 'Cavendish' group as shown in Figure 1. In the past, germplasm repositories relied on morphological traits, the majority of which were described by Simmonds and Shepherd (1955) to determine genomic composition. However, plant morphology can be influenced by the environment and the visual descriptors are sometimes subjective, which may have led to mistakes in genomic composition determination in the past.

The USDA ARS Tropical Agriculture Research Station (TARS) in Mayagüez, Puerto Rico, is responsible for the maintenance of the *Musa* spp. collection, which consists of bananas, plantains and cooking bananas of local and international selections and hybrids from interna-

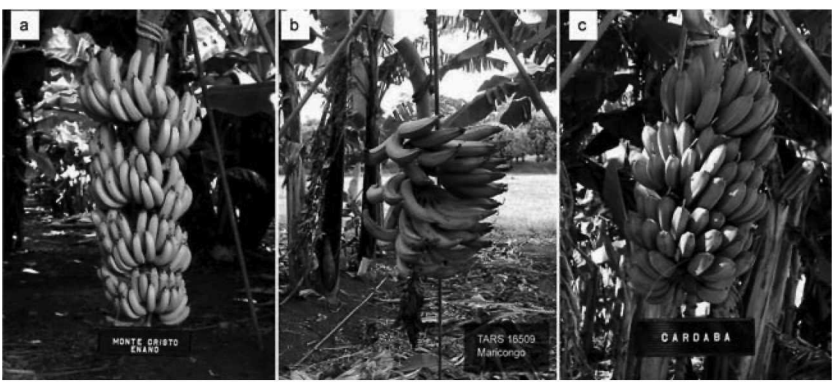

FIGURE 1. Bunch phenotype for three representative groups of *Musa* spp. in the field collection. a) Cavendish type (AAA); b) False-horn type plantain (AAB); c) Cooking banana (ABB).

tional breeding programs. Some accessions have known genomic composition, but many accessions are yet to be determined. The random amplified polymorphic DNA technique (RAPD) and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) have been used extensively in research for genetic fingerprinting (Howell et al., 1994). Pillay et al. (2000; 2006) utilized the RAPD technique to distinguish plants containing pure A or B genomes as well as to determine whether genomic hybrids existed. In these experiments the RAPD technique could also determine the B genome copy number. Pillay et al. (2000) screened a large number of RAPD primers from Operon Technologies (Alameda, CA, USA) and three particular primers (A17, A18, D10) were identified that were useful in distinguishing the genomic composition in accessions of *Musa* spp. The PCR-RFLP technique has been used in genetic fingerprinting of fungi (Gomez et al., 2002), bacteria (Ormeno-Orillo et al., 2006), nematodes (Uehara et al., 2005) as well as of plants (Ge et al., 2005). Nwakanma et al. (2003) described the use of the PCR-RFLP technique for distinguishing genomic composition in *Musa* spp. The technique is based on the differences observed in the restriction patterns of the internal transcribed spacers (ITS) region of the nuclear ribosomal DNA genes.

Flow cytometry is a tool that analyzes cells and particles in a liquid stream. Application of flow cytometry to plant breeding and germplasm characterization includes ploidy determination of breeding parents and available germplasm, identification of ploidy chimerism, genome size determination, and offspring screening for aneuploid, mixoploid, and other unusual ploidy compositions (Eeckhaut et al., 2005). Because of the fact that suspensions of nuclei are prepared by finely chopping fresh tissue into an isolation and staining buffer, large numbers of individuals can be quickly and accurately analyzed (Galbraith et al., 1983; Pillay et al., 2000; 2006), an advantage compared to histological chromosome counting.

The objectives of the current research were 1) to utilize flow cytometry and molecular fingerprinting techniques to determine the genomic composition for the 135 *Musa* spp. accessions held in the USDA-ARS TARS collection; 2) to determine whether any propagation errors (i.e., mislabeling, planting mistakes) had occurred in the collection; and 3) to compare the ploidy level and genomic composition results of this research with that found in the previously existing literature and databases.

MATERIALS AND METHODS

Plant material / DNA extraction. Plant material evaluated included all 135 *Musa* spp. accessions maintained at the USDA-ARS TARS at

Isabela, Puerto Rico. A single sample was evaluated for 'Saba', an accession provided from collaborators at the University of Puerto Rico Experimental Station in Corozal, Puerto Rico.

Musa species in the evaluation include *M. acuminata*, *M. balbisiana* and their hybrids. Two other species of the *Musa* spp. collection, *M. velutina* and *M. ornata*, members of the Rhodochlamys section, were included as controls for comparisons. Young leaves from three separate plants for each accession were collected in the field and brought to the laboratory for further processing. DNA extraction was carried out with a Fast DNA® SPIN Kit[6] (MP Biomedicals, Irvine, CA, USA). DNA quality and quantity was measured with a NanoDrop® ND-1000 UV-Vis spectrophotometer (NanoDrop, Wilmington, DE, USA).

Ploidy Analysis. Approximately 0.5 cm^2 of tissue was excised from leaves of in vitro-grown *Musa* spp. plantlets. Nuclei were released by finely chopping the tissue with a razor blade into 400 µl of CyStain UV Ploidy precise P extraction buffer (code No. 05-5002, Partec GmbH, Münster, Germany). The chopped tissue and released nuclei were incubated for two minutes, and filtered through a Partec CellTrics 50-µm disposable filter (code No. 04-0041-2317). This was followed by the addition of 1.6 ml of CyStain UV precise P staining buffer, and then analyzed in a Partec Ploidy Analyzer I flow cytometer. Prior to running *Musa* spp. samples, the flow cytometer was calibrated by using the cultivar Kirkirnan as a validated diploid *Musa* genotype as shown in Figure 2. Following calibration with the 2n accession Kirkirnan, the histogram peak was set at 100 for the diploid accessions, centering histogram peaks for triploid accessions around 150, and for the tetraploid accessions around 200.

RAPD markers. The PCR amplification reaction for the RAPD technique followed general steps described by Pillay et al. (2000; 2006). The reaction was carried out in a 25.0-µl volume, consisting of 2.0 µl DNA (100 ng/µl), 14.75 µl of ddH_2O, 0.5 µl of dNTP (each 2.5 mM), 2.5 µl 10X PCR buffer (Promega, Madison, WI, USA), 2.0 µl of $MgCl_2$, 3.0 µl of primer (10 mM) and 0.25 µl of Taq polymerase (5 Units/µl) (Promega, Madison, WI, USA). Amplification was performed in a BIORAD iCycler™ thermal cycler (Biorad, Hercules, CA, USA) with the following steps: an initial 3-min denaturation at 94° C followed by 35 cycles of 50 sec at 94° C, 50 sec at 40° C, and 1.5 min at 72° C, with a final extension step of 7 min at 72° C. Approximately 15 µl of the amplification product was separated in a 1.2% agarose gel in 1X TBE (Tris/Borate/EDTA)

[6]Mention of trade names or commercial products in this article are solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or the University of Puerto Rico.

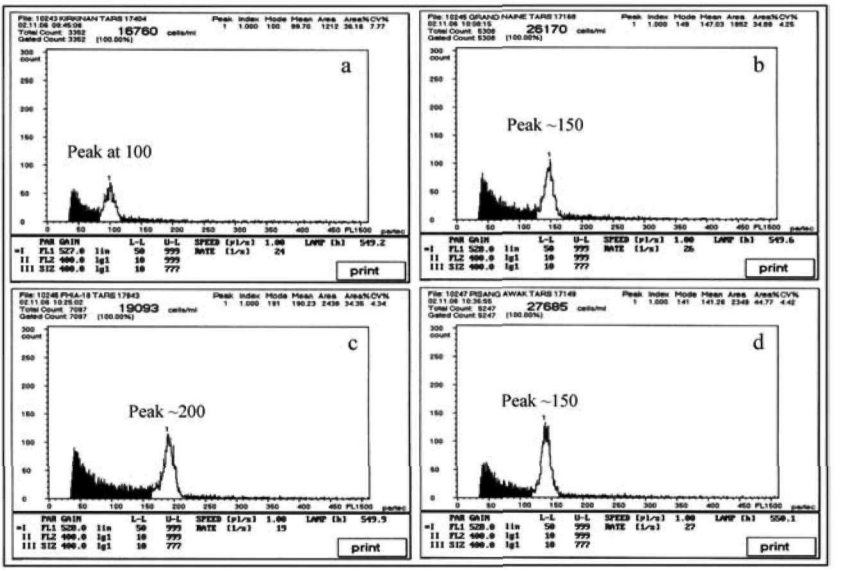

FIGURE 2. Representative histograms for four *Musa* spp. accessions. A) 'Kirkirnan' = 2N was used to standardize the flow cytometer with a peak centered around 100; b) 'Dwarf Cavendish' = 3N ~150; c) 'FHIA-18' = 4N~200; and d) 'Pisang Awak' showing that it is a triploid (3N) accession (peak centered around 150).

buffer. A 100-bp molecular weight marker (Promega, Madison, WI, USA) was included in all agarose gels. The gels were stained with ethidium bromide and digitally photographed on an ultraviolet light transilluminator. The resulting fingerprints were assessed and scored manually.

PCR-RFLP markers. General steps for the PCR-RFLP amplification reaction were carried out as described by Nwakanma et al. (2003). The PCR step was carried out in a 50-µl reaction consisting of 2 µl DNA (~100 ng/µl), 31.6 µl of ddH_2O, 4 µl of dNTP (each 2.5 mM), 5 µl of 10X PCR buffer (Promega, Madison, WI, USA), 2 µl of $MgCl_2$, 2.5 µl each of ITS-L and ITS-4 primers (10 mM) and 0.4 µl of Taq polymerase (5 Units/µl) (Promega, Madison, WI, USA). Amplification of DNA samples for the PCR-RFLP technique was performed in a BIORAD iCycler™ thermal cycler (Biorad, Hercules, CA, USA) with the following steps: an initial 4-min denaturation at 94° C followed by 35 cycles of 30-sec denaturation at 94° C, 30-sec annealing at 50° C, 1-min extension at 72° C, with a final 7-min incubation at 72° C. Approximately 5 µl of the amplification product was separated and visualized on a 1.0% agarose gel in 1X TBE buffer. A molecular weight marker of a 100-bp ladder

(Promega, Madison, WI, USA) was included in all gels for size comparisons. In a second step, and after verification of successful amplification, the amplified fragments were restricted with the enzyme *Rsa*I in a 60-µl reaction. The restriction reaction was carried out with the 45 µl of the PCR reaction and 15 µl of a restriction reaction consisting of 7 µl of ddH_2O, 6 µl of 10X RE buffer, 1 µl of BSA and 1 µl of the *Rsa*I enzyme (Promega, Madison, WI, USA) (10 Units/µl). Approximately 30 µl of the product was loaded and separated in a 3.0% gel. Genomic composition was determined visually from gels that had been stained with ethidium bromide and digitally photographed on an ultraviolet light transilluminator.

RESULTS AND DISCUSSION

Results for the flow cytometry studies showed that both *M. ornata* and *M. velutina* are diploid species (Table 1). Of the remaining 133 accessions, excluding 'Saba', the number that were diploid, triploid, and tetraploid was 16, 97, and 20, respectively.

Although the fingerprints generated for the three DNA samples from a given accession were identical and could be distinguished from the samples of the other accessions being evaluated, the RAPD technique has long been criticized because of its lack of reproducibility and consistent results (Crouch et al., 2000). This discrepancy was true in our evaluations, where all three of the RAPD primers identified by Pillay et al. (2000) as useful in determining genomic composition of *Musa* spp. failed to amplify the specific marker fragments that distinguished the different genomic groups (data not shown).

Because of the lack of reproducibility with the RAPD technique, a second more robust technique, PCR-RFLP markers, described by Nwakanma et al. (2003) was adopted. With this technique, PCR amplification of the internal transcribed spacers (ITS) region of the nuclear rDNA gene of *Musa* spp. produced a 700-bp fragment. This fragment was then restricted with a specific restriction enzyme, *Rsa*I, to produce unambiguous genome fingerprint profiles. The PCR-RFLP technique was able to distinguish between the different species and their hybrids. Results obtained showed that our *M. balbisiana* accession produced a different fingerprint profile with four fragments: 350-bp, 180-bp, 120-bp, and 50-bp as seen in the example for 'Tani' (Figure 3). In contrast, *M. acuminata* accessions produced fingerprint profiles consisting of three fragments, of sizes 530-bp, 120-bp, and 50-bp as shown for the examples of 'Pisang Klutuk Wulung', 'Kirkirnan' and 'Dwarf Cavendish' in Figure 3. Hybrid accessions showed a shared fingerprint profile consisting of all fragment sizes, 530-bp, 350-bp, 180-bp, 120-bp, and 50-bp.

TABLE 1.— *Ploidy analysis and genome composition for* Musa *spp. accessions curated by the USDA ARS Tropical Agriculture Research Station (TARS), Mayagüez, Puerto Rico.*

					Previously reported[1]		This study[2]	
	TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
Ornamental species								
	17951	Flowering banana	*Musa*	*ornata*	—	—	2	—
	18044	Velvet Pink	*Musa*	*velutina*	—	—	2	—
Diploid BB								
	18046	Tani	*Musa*	*balbisiana*	2	BB	2	BB
Diploid AA								
	17413	Datil La Lima	*Musa*	*acuminata*	—	—	2	AA
	18021	Fenjiao	*Musa*	*acuminata*	3	ABB	2	AA
	17399	Hapai	*Musa*	*acuminata*	2	AA	2	AA
	17412	Katual Valunair	*Musa*	*acuminata*	2	AA	2	AA
	17404	Kirkirnan	*Musa*	*acuminata*	2	AA	2	AA
	17411	Kuspaka (PNG-219)	*Musa*	*acuminata*	2	AA	2	AA
	17390	Maia Hapai	*Musa*	*acuminata*	2	AA	2	AA
	17425	Mosslin	*Musa*	*acuminata*	—	—	2	AA

[1]Reported ploidy and genomic composition by identification of matching accession names (from Ploetz et al., 2007 and Bioversity International's MGIS database http://195.220.148.3:8013/mgis_2/homepage.htm [last accessed 11/28/2008]).

[2]Ploidy and genomic composition determined in this study by flow cytometry and use of PCR-RFLP makers.

[3]Mixoploid mutant of 'Tapo', a diploid *M. acuminata* (AA) with red foliage.

[4] Pillay et al. (2006) reports 'Pisang Awak' as a tetraploid hybrid.

[5]'Saba' University of Puerto Rico Experiment Station, Corozal, PR (not part of the USDA ARS Tropical Agriculture Research Station collection).

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					Previously reported[1]		This study[2]	
	TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
	17379	Niño Comun	*Musa*	*acuminata*	2	AA	2	AA
	17142	Niño Enano	*Musa*	*acuminata*	2	AA	2	AA
	18045	P. Klutuk Wulung	*Musa*	*acuminata*	2	BB	2	AA
	17403	Patupi (PNG-225)	*Musa*	*acuminata*	2	AA	2	AA
	18057	Siam Ruby[3]	*Musa*	*acuminata*	2	AA	2 (4)	AA
	17382	Tuu-Ghia	*Musa*	*acuminata*	2	AA	2	AA
Triploid AAA								
	17164	1-A	*Musa*	*acuminata*	—	—	3	AAA
	17125	2-A	*Musa*	*acuminata*	—	—	3	AAA
	17154	3-A	*Musa*	*acuminata*	—	—	3	AAA
	17135	5-A	*Musa*	*acuminata*	—	—	3	AAA
	17130	6-A	*Musa*	*acuminata*	—	—	3	AAA
	17159	8-A	*Musa*	*acuminata*	—	—	3	AAA
	17167	9-A	*Musa*	*acuminata*	—	—	3	AAA
	17152	10-A	*Musa*	*acuminata*	—	—	3	AAA

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				Previously reported[1]		This study[2]	
TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
17138	1-C-2	*Musa*	*acuminata*	4	AAAA	3	AAA
17137	1-R-2, 500	*Musa*	*acuminata*	—	—	3	AAA
17131	2-R-2, 500	*Musa*	*acuminata*	—	—	3	AAA
17141	3-R-2, 500	*Musa*	*acuminata*	—	—	3	AAA
17392	Cuban Red	*Musa*	*acuminata*	3	AAA	3	AAA
16525	Dwarf Cavendish	*Musa*	*acuminata*	3	AAA	3	AAA
17402	Dwarf-Valery	*Musa*	*acuminata*	3	AAA	3	AAA
17143	Enano Gigante	*Musa*	*acuminata*	—	—	3	AAA
17162	Giant Cavendish	*Musa*	*acuminata*	3	AAA	3	AAA
17163	Giant Governor	*Musa*	*acuminata*	3	AAA	3	AAA
17175	Gigante Blanco	*Musa*	*acuminata*	—	—	3	AAA
17168	Gran Nain	*Musa*	*acuminata*	3	AAA	3	AAA
17155	Gros Michel	*Musa*	*acuminata*	3	AAA	3	AAA
17840	Guaran-enano	*Musa*	*acuminata*	3	AAA	3	AAA
17177	Guineo doble	Musa	acuminata	—	—	3	AAA

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				Previously reported[1]		This study[2]	
TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
17426	Guineo Enano	*Musa*	*acuminata*	3	AAA	3	AAA
17153	Highgate	*Musa*	*acuminata*	3	AAA	3	AAA
17396	Igcpoca	*Musa*	*acuminata*	—	—	3	AAA
17409	Ignamico	*Musa*	*acuminata*	—	—	3	AAA
17144	Lacatan	*Musa*	*acuminata*	3	AAA	3	AAA
17172	Mahoe	*Musa*	*acuminata*	3	AAA	3	AAA
17417	Monte Cristo	*Musa*	*acuminata*	3	AAA	3	AAA
17428	Monte Cristo Enano	*Musa*	*acuminata*	3	AAA	3	AAA
17148	Morado	*Musa*	*acuminata*	3	AAA	3	AAA
17178	Morado enano	*Musa*	*acuminata*	3	AAA	3	AAA
17408	Nchumbahoka	*Musa*	*acuminata*	3	AAA	3	AAA
17145	Pisang Kelat	*Musa*	*acuminata*	3	AAB	3	AAA
17160	Paggi	*Musa*	*acuminata*	—	—	3	AAA
17423	Royal P. & Vol. Musa	*Musa*	*acuminata*	—	—	3	AAA
17385	Sc-2T	*Musa*	*acuminata*	—	—	3	AAA

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					Previously reported[1]		This study[2]	
	TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
	17395	Taiwanese somaclone	*Musa*	*acuminata*	—	—	3	AAA
	17405	Tigua (PNG-265)	*Musa*	hybrid	3	AAB	3	AAA
	17158	Valery	*Musa*	*acuminata*	3	AAA	3	AAA
	17150	Verdin	*Musa*	*acuminata*	—	—	3	AAA
	17414	Williams	*Musa*	*acuminata*	3	AAA	3	AAA
	17844	Yangambi km 5	*Musa*	*acuminata*	3	AAA	3	AAA
	17169	Ziv	*Musa*	*acuminata*	—	—	3	AAA
Triploid AAB 'True plantains'								
	17181	African Rhino	*Musa*	hybrid	—	—	3	AAB
	16512	Chinga	*Musa*	hybrid	—	—	3	AAB
	16506	Colombian dwarf	*Musa*	hybrid	—	—	3	AAB
	16505	Common dwarf	*Musa*	hybrid	—	—	3	AAB
	16510	Common Harton	*Musa*	hybrid	—	—	3	AAB
	16513	Congo-300	*Musa*	hybrid	—	—	3	AAB
	17179	Corozal – Selection 25	*Musa*	hybrid	—	—	3	AAB

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					Previously reported[1]		This study[2]	
	TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
	16508	Dominican dwarf	*Musa*	hybrid	—	—	3	AAB
	17180	Dominico Harton	*Musa*	hybrid	3	AAB	3	AAB
	16507	Dwarf Superplantain	*Musa*	hybrid	—	—	3	AAB
	17813	False Horn Dom. Red	*Musa*	hybrid	—	—	3	AAB
	17816	French Dominican Red	*Musa*	hybrid	—	—	3	AAB
	17825	French dwarf	*Musa*	hybrid	3	AAB	3	AAB
	17814	Harton Select. Chago	*Musa*	hybrid	—	—	3	AAB
	16516	Laknau P.I. 23472	*Musa*	hybrid	3	AAB	3	AAB
	16515	Laknau P.I. 23479	*Musa*	hybrid	3	AAB	3	AAB
	16511	Maiden plantain	*Musa*	hybrid	3	AAB	3	AAB
	16509	Maricongo	*Musa*	hybrid	3	AAB	3	AAB
	16514	Plant. w/o male flower	*Musa*	hybrid	—	—	3	AAB
	17815	Tall Superplantain	*Musa*	hybrid	—	—	3	AAB
Triploid AAB					—	—		
	17134	Antigua Finger Rose	*Musa*	hybrid	—	—	3	AAB

[1]Reported ploidy and genomic composition by identification of matching accession names (from Ploetz et al., 2007 and Bioversity International's MGIS database http://195.220.148.3:8013/mgis_2/homepage.htm [last accessed 11/28/2008]).

[2]Ploidy and genomic composition determined in this study by flow cytometry and use of PCR-RFLP makers.

[3]Mixoploid mutant of 'Tapo', a diploid *M. acuminata* (AA) with red foliage.

[4] Pillay et al. (2006) reports 'Pisang Awak' as a tetraploid hybrid.

[5]'Saba' University of Puerto Rico Experiment Station, Corozal, PR (not part of the USDA ARS Tropical Agriculture Research Station collection).

TABLE 1.—(Continued) *Ploidy analysis and genome composition for* Musa *spp. accessions curated by the USDA ARS Tropical Agriculture Research Station (TARS), Mayagüez, Puerto Rico.*

					Previously reported[1]		This study[2]	
	TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
	18026	Dare	*Musa*	hybrid	3	AAB	3	AAB
	17176	Golden Pillow	*Musa*	hybrid	—	—	3	AAB
	17824	Hua moa 'Popoulu'	*Musa*	hybrid	3	AAB	3	AAB
	17378	Hy Brazilian	*Musa*	hybrid	3	AAB	3	AAB
	17407	Mai'a Maoli Eka	*Musa*	hybrid	3	AAB	3	AAB
	17136	Manzano	*Musa*	hybrid	3	AAB	3	AAB
	17171	Mysore	*Musa*	hybrid	3	AAB	3	AAB
	17140	Pirineo	*Musa*	hybrid	—	—	3	AAB
	16522	Rajapuri	*Musa*	hybrid	3	AAB	3	AAB
	17410	Señorita	*Musa*	hybrid	—	—	3	AAB
	17992	Thousand fingers	*Musa*	hybrid	3	ABB	3	AAB
	18060	Ubok Iba	*Musa*	hybrid	3	AAB	3	AAB
Triploid ABB 'Cooking bananas'								
	18018	Blue Torres S. Island	*Musa*	hybrid	3	ABB	3	ABB
	18019	Bom	*Musa*	hybrid	3	ABB	3	ABB

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TABLE 1.—(Continued) *Ploidy analysis and genome composition for* Musa *spp. accessions curated by the USDA ARS Tropical Agriculture Research Station (TARS), Mayagüez, Puerto Rico.*

				Previously reported[1]		This study[2]	
TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
18020	Cacambou	*Musa*	hybrid	3	ABB	3	ABB
17822	Cardaba - Honduras	*Musa*	hybrid	3	ABB	3	ABB
17128	Dwarf Chamaluco	*Musa*	hybrid	3	ABB	3	ABB
17397	Dwarf Orinoco	*Musa*	hybrid	—	—	3	ABB
18022	Fougamou 1	*Musa*	hybrid	3	ABB	3	ABB
18023	Gipungusi	*Musa*	hybrid	—	ABB	3	ABB
17146	Mafafo de Adjuntas	*Musa*	hybrid	—	—	3	ABB
17126	Mafafo de Puerco	*Musa*	hybrid	—	—	3	ABB
17133	Mafafo Dominicano	*Musa*	hybrid	—	—	3	ABB
17424	Paka	*Musa*	hybrid	2	AA	3	ABB
17819	Pelipita (Colombia)	*Musa*	hybrid	3	ABB	3	ABB
17818	Pelipita (Costa Rica)	*Musa*	hybrid	3	AAB	3	ABB
17149	Pisang Awak	*Musa*	hybrid	3[4]	AAB	3	ABB
17132	Pisang siguatepeque	*Musa*	hybrid	3	ABB	3	ABB
17157	Poni	*Musa*	hybrid	—	—	3	ABB

[1]Reported ploidy and genomic composition by identification of matching accession names (from Ploetz et al., 2007 and Bioversity International's MGIS database http://195.220.148.3:8013/mgis_2/homepage.htm [last accessed 11/28/2008]).

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TABLE 1.—(Continued) *Ploidy analysis and genome composition for* Musa *spp. accessions curated by the USDA ARS Tropical Agriculture Research Station (TARS), Mayagüez, Puerto Rico.*

					Previously reported[1]		This study[2]	
	TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
	17173	Praying hands	*Musa*	hybrid	—	—	3	ABB
	—	Saba[5]	*Musa*	hybrid	3	ABB/ BBB	3	ABB
Synthetic hybrids								
	18049	TMB2x 9128-3	Synthetic	hybrids	2	AA	2	AA
	18048	Pita-16	Synthetic	hybrids	—	—	3	AAB
	17165	2390	Synthetic	hybrids	4	AAAA	4	AAAA
	18056	CRBP 39	Synthetic	hybrids	4	AAAB	4	AAAB
	17760	FHIA-02 (Mona Lisa)	Synthetic	hybrids	4	AAAB	4	AAAA
	17761	FHIA-03	Synthetic	hybrids	4	AABB	4	AABB
	18058	FHIA-17	Synthetic	hybrids	4	AAAA	4	AAAA
	17843	FHIA-18 (Bananza)	Synthetic	hybrids	4	AAAB	4	AAAB
	17820	FHIA-21	Synthetic	hybrids	4	AAAB	4	AAAB
	18059	FHIA-23	Synthetic	hybrids	4	AAAA	4	AAAA
	18047	SH-3640 (High Noon)	Synthetic	hybrids	4	AAAB	4	AAAB

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TABLE 1.—(Continued) *Ploidy analysis and genome composition for* Musa *spp. accessions curated by the USDA ARS Tropical Agriculture Research Station (TARS), Mayagüez, Puerto Rico.*

				Previously reported[1]		This study[2]	
TARS#	Accession/cultivar	Genus	Species	Ploidy	Genomic Composition	Ploidy	Genomic Composition
18050	PA03-22	Synthetic	hybrids	4	AAAB	4	AAAB
18051	PA 12-03	Synthetic	hybrids	4	AAAB	4	AAAB
18052	PV03-44	Synthetic	hybrids	4	AAAB	4	AAAB
18053	PV 42-53	Synthetic	hybrids	—	—	4	AAAB
18054	PV 42-81	Synthetic	hybrids	—	—	4	AAAB
18055	PV 42-320	Synthetic	hybrids	—	—	4	AAAB
17841	TMB × 1378	Synthetic	hybrids	—	—	4	AABB
17842	TMB × 5295-1	Synthetic	hybrids	—	—	4	AAAB
17817	TMP × 1621-1	Synthetic	hybrids	—	—	4	AAAB
17821	TMP × 4479-1	Synthetic	hybrids	—	—	4	AAAB
17823	TMP × 7152-2	Synthetic	hybrids	—	—	4	AAAB

[1]Reported ploidy and genomic composition by identification of matching accession names (from Ploetz et al., 2007 and Bioversity International's MGIS database http://195.220.148.3:8013/mgis_2/homepage.htm [last accessed 11/28/2008]).

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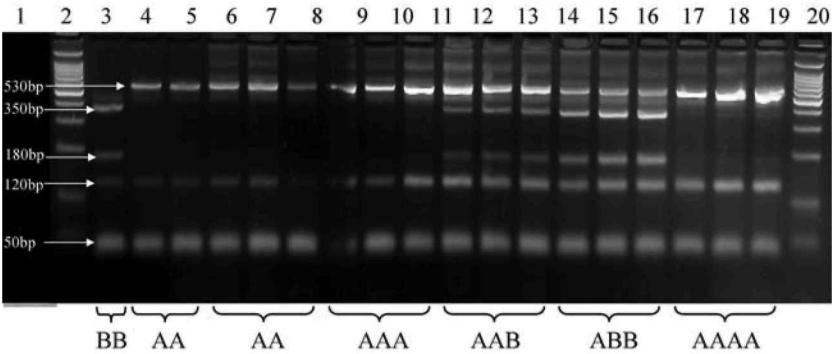

Lane #		Description
1, 20	—	100 bp marker
2	—	'Tani'; BB
3, 4	—	'Pisang Klutuk Wulung'; AA
5,6,7	—	'Kirkirnan'; AA
8,9,10	—	'Dwarf Cavendish'; AAA
11,12,13	—	'Maricongo'; AAB
14,15,16	—	'Mafafo Dominicano'; ABB
17,18,19	—	'FHIA - 02'; AAAA

FIGURE 3. Gel image depicting typical PCR-RFLP fingerprint profiles for seven *Musa* spp. accessions differing in ploidy and genomic composition. Note: For the lanes with a 100-bp marker, the 500 bp band appears more intense than other bands.

Musa velutina and *M. ornata* produced slightly larger amplification products, as well as fingerprint profiles discernible from those for the *M. balbisiana* and *M. acuminata* and their hybrids (data not shown). A quantitative effect of the specific diagnostic fragment intensities for the *M. acuminata* and *M. balbisiana* genomes in the PCR-RFLP technique was also observed; as the genome copy number increased so did the amplified fragment intensities. Therefore, accessions with more than one copy of the A or B genome showed stronger fragment intensities than those with only one set of either genome. In addition, a trend was observed where accessions with a higher ploidy level showed overall stronger fragment intensities than those with lower ploidy.

On the basis of the molecular fingerprinting techniques and ploidy analysis, results showed the collection consisted of diploids, triploids and tetraploids. The number of diploid pure *M. acuminata* (AA) accessions was 14; triploid pure *M. acuminata* (AAA) accessions was 45; triploid hybrid accessions or "true plantains" (AAB) was 20; other triploids (AAB) was 13; and triploid hybrid accessions "cooking bananas" (ABB) was 18 (excluding 'Saba'). A total of 22 synthetic hybrids with di- (2),

tri- (3), and tetra- (4) ploidy levels are also included in the collection (Table 1). A single pure *M. balbisiana* accession (BB) was identified in the collection (Table 1).

Some disagreement was identified in the literature with regards to ploidy level and genomic composition for several cultivars and germplasm accessions. For example, 'Pisang Awak' was found to be a triploid 'cooking banana' (ABB) (Figure 2), a report which differs with a recent description by Pillay et al. (2006) stating that 'Pisang Awak' is a tetraploid hybrid. Several other accessions evaluated in the collection did not match information contained in databases or in published information with regard to ploidy and genomic composition. For example, 'Pisang Kelat' is commonly listed in the literature as having a hybrid genomic composition (AAB); however, the three plants screened with the PCR-RFLP technique all belonged to the triploid *M. acuminata* (AAA) group. Also, 'Tigua' has been reported as a "Pacific Plantain" in the 'Iholena' subgroup (Ploetz et al., 2007), but in our results the plant's fingerprint matches that of a *M. acuminata* triploid (AAA). When an image of the bunch/fruit phenotype for the local 'Tigua' accession was compared to an image for an accession with the same name in Bioversity International's *Musa* Germplasm Information System (MGIS) database, the images did not look alike. Nevertheless, a description from Ploetz et al. (2007) matches closely the phenotype of the currently held accession. Database records indicate that 'Pisang Klutuk Wulung' belongs to the *M. balbisiana* species, but the TARS accession with this name is *M. acuminata* on the basis of the fingerprint profile (Figure 3) as well as observed phenotypic traits. This accession was recently acquired from Bioversity International's International Transit Center (ITC) for *Musa*. Data from three DNA samples from the three field plants tested for 'Mai'a Maoli Eka' found that two of the three plants for this accession belong to the Pacific plantain or Iholena subgroup (AAB), which is the correct genomic composition according to database information, but the third plant exhibited a PCR-RFLP profile of a *M. acuminata* (AAA) type. The 'Saba' accession has been reported to be a triploid *M. balbisiana* (BBB) (Rowe, 1987; MGIS database - http://195.220.148.3:8013/mgis_2/homepage.htm); in our studies it was found to be a triploid, but of a hybrid "cooking banana-ABB" genotype. 'Paka', an accession with a hybrid fingerprint profile corresponding to a "cooking banana" or ABB genomic composition, when analyzed with the flow cytometer, was a diploid. This discrepancy was quickly resolved when the field plants were processed for a second time for the ploidy analysis. It appears as though the tissue culture plantlet used for the initial ploidy analysis did not belong to the same accession and corresponded to a mistaken diploid (AA) accession. Two additional accessions, 'Fen-

jiao' and '1-C-2', differed in reported and observed ploidy levels/genomic composition. Some ambiguity was encountered in the literature with regards to the genomic composition for 'FHIA-02' which in our studies was identified as being a tetraploid hybrid with all genome copies arising from *M. acuminata* (Figure 3). Several of the discrepancies identified in the *Musa* spp. collection accessions may have been caused by human error in past management of the collection. The sheer number of synonymous and local common names attributed to *Musa* spp. genotypes worldwide may also play a significant role in the inconsistencies identified (see Ploetz et al., 2007).

The genomic composition for several of the synthetic hybrids, especially those of the tetraploid accessions, was difficult to determine. The two diagnostic maker fragments of the *M. balbisiana* genome (350 and 180 bp) were difficult to detect. The fragment's weak appearance might have been due to insufficient availability of PCR reagents (i.e., primers, dNTPs), lack of optimal conditions during PCR amplification or possible lack of primer efficiency due to base pair mismatches. Since the 700bp ITS region was being amplified for four fragments or genome copies (tetraploids) it is possible that the number of A genome copies outnumbered the number of B genome copies and therefore this might have had an influence in the intensity at which diagnostic fragments were amplified and detected for these accessions.

Although flow cytometry and PCR-RFLP markers were shown to be valuable tools for the determination of ploidy, genomic composition, and for elimination of propagation mistakes within the locally maintained germplasm collection, this resolution allows only for classification of accessions into general groups and does not distinguish among accessions within these groups. Other molecular marker techniques, such as microsatellites, could be more useful in distinguishing among accessions within specific genomic compositions (i.e., among 'Cavendish' types). This method would allow for identification of possible propagation mistakes within plant groups, provide a unique fingerprint profile for each accession, as well as provide an estimate of genetic diversity maintained in the collection. Current efforts are focused on identification of microsatellite markers with potential use in *Musa* spp. fingerprinting.

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