Identification of RAPD markers linked to A and B genome sequences in *Musa* L.

M. Pillay, D.C. Nwakanma, and A. Tenkouano

Abstract: Plantains and bananas (*Musa* spp. sect. *eumusa*) originated from intra- and interspecific hybridization between two wild diploid species, *M. acuminata* Colla. and *M. balbisiana* Colla., which contributed the A and B genomes, respectively. Polyploidy and hybridization have given rise to a number of diploid, triploid, and tetraploid clones with different permutations of the A and B genomes. Thus, dessert and highland bananas are classified mainly as AAA, plantains are AAB, and cooking bananas are ABB. Classification of *Musa* into genomic groups has been based on morphological characteristics. This study aimed to identify RAPD (random amplified polymorphic DNA) markers for the A and B genomes. Eighty 10-mer Operon primers were used to amplify DNA from *M. acuminata* subsp. *burmannicoides* clone 'Calcutta 4' (AA genomes) and *M. balbisiana* clone 'Honduras' (BB genomes). Three primers (A17, A18, and D10) that produced unique genome-specific fragments in the two species were identified. These primers were tested in a sample of 40 genotypes representing various genome combinations. The RAPD markers were able to elucidate the genome composition of all the genotypes. The results showed that RAPD analysis can provide a quick and reliable system for genome identification in *Musa* that could facilitate genome characterization and manipulations in breeding lines.

Key words: banana and plantain, A and B genomes, genomic groups, RAPD markers.

Résumé : Le bananier-plantain et le bananier (*Musa* spp. sect. *eumusa*) proviennent d'hybridations intra- et interspécifiques entre deux espèces sauvages diploïdes, *M. acuminata* Colla. et *M. balbisiana* Colla., lesquelles ont contribué les génomes A et B respectivement. La polyploïdie et l'hybridation ont produit une gamme de clones diploïdes, triploïdes et tétraploïdes présentant diverses permutations des génomes A et B. Ainsi, les bananiers des déserts ou des hauts plateaux sont classifiés principalement comme étant AAA, les bananier-plantains sont AAB et les bananes à cuire sont ABB. La classification de ces plantes du genre *Musa* en divers groupes génomiques s'est faite sur la base de caractères morphologiques. La présente étude visait à identifier des marqueurs RAPD (ADN polymorphe amplifié au hasard) pour les génomes A et B. Quatre-vingt amorces Operon ont été utilisées pour amplifier l'ADN du *M. acuminata* spp. *burmannicoides* clone 'Calcutta 4' (génome AA) et celui du *M. balbisiana* clone 'Honduras' (génome BB). Trois amorces (A17, A18 et D10) amplifiant des régions uniques à chacun des génomes ont été identifiées. Ces amorces ont été employées sur un échantillon de 40 génotypes représentant diverses combinaisons génomiques. Les marqueurs RAPD ont été capables d'élucider la composition génomique de tous les génotypes. Les résultats ont montré que l'analyse RAPD peut être utilisée pour identifier de façon fiable et rapide la composition génomique chez le genre *Musa*. Ceci pourrait ainsi faciliter la caractérisation et la manipulation des génomes dans le cadre de travaux d'amélioration.

Mots clés : bananier et bananier-plantain, génomes A et B, groupes génomiques, RAPD.

[Traduit par la Rédaction]

Introduction

Plantains and bananas (*Musa* spp. L.) originated from intra- and interspecific hybridization between two wild diploid species; *M. acuminata* Colla. and *M. balbisiana* Colla. The two species contributed the A and B genomes, respectively (Simmonds 1995). Further evolution in *Musa* occurred through polyploidization and the accumulation of somatic mutations (Stover and Simmonds 1987).

Bananas and plantains have a basic chromosome number of $x = 11$, with 22 (diploid), 33 (triploid), and 44 (tetraploid) chromosomes (Stover and Simmonds 1987). Various *Musa* genomic groups consist of either one or different permutations of the basic genomes. Thus, cultivated dessert and east African Highland bananas are classified mainly as AAA, plantains are AAB, and cooking bananas are ABB. Other combinations including AB, AAAB, AABB, and ABBB also exist, which occur naturally or are produced by artificial hybridization (Stover and Simmonds 1987). The ancestral species *M. acuminata* and *M. balbisiana* evolved in vastly different environments and contributed several agronomic traits towards the present genetic composition of the various *Musa* clones. For example, genes for hardiness, drought tol-

Corresponding Editor: J.P. Gustafson.

Received July 9, 1999. Accepted May 18, 2000. Published on the NRC Research Press web site on August 25, 2000.

M. Pillay[1], D.C. Nwakanma, and A. Tenkouano. Plantain and Banana Improvement Project, International Institute of Tropical Agriculture (IITA), High Rainfall Station, Onne, PMB 008, Nchia Eleme, Rivers State, Nigeria.

[1]Author to whom all correspondence should be addressed at the International Institute of Tropical Agriculture (IITA), Co L.W. Lambourn & Co., Carolyn House, 26 Dingwall Road, Croydon CR9 3EE, England (e-mail: M.Pillay@satmail.bt.com).

Table 1. List of landraces used to verify RAPD markers for A and B genomes in *Musa*.

Genotype	Genome group	Ploidy	Diagnostic RAPD bands*			
			A17/D10	$A18_{200}$	$A18_{250}$	$A18_{300}$
M. balbisiana Hnd	BB	$2n = 22$		X	X	X
M. balbisiana Butohan 1	BB	$2n = 22$		X	X	X
M. balbisiana Butohan 2	BB	$2n = 22$		X	X	X
M. balbisiana 1-63	BB	$2n = 22$		X	X	X
M. balbisiana 1085	BB	$2n = 22$		X	X	X
M. balbisiana MPL	BB	$2n = 22$		X	X	X
M. balbisiana Los Banos	BB	$2n = 22$		X	X	X
M. balbisiana Singapuri	BB	$2n = 22$		X	X	X
M. balbisiana Tani	BB	$2n = 22$		X	X	X
M. balbisiana Eti kehel	BB	$2n = 22$		X	X	X
'Monthan Saba'	ABB/BBB	$2n = 33$	X	X	X	X
'Bluggoe'	ABB	$2n = 33$	X	X	X	X
'Cardaba'	ABB	$2n = 33$	X	X	X	X
'Klue Tiparot'	ABBB/ABB	$2n = 44/33$	X	X	X	X
'Kisube'	AB	$2n = 22$	X		X	X
'Kamaramasenge'	AB	$2n = 22$	X		X	X
'Bobby tannap'	AAB	$2n = 33$	X		X	X
'Obino l'Ewai'	AAB	$2n = 33$	X		X	X
'Ngern'	AAAB	$2n = 44$	X		X	X
'Oura da mate'	AAAB	$2n = 44$	X		X	X
M. acuminata C4	AA	$2n = 22$	X			
M. acuminata Panang	AA	$2n = 22$	X			
M. acuminata Zebrina	AA	$2n = 22$	X			
'Pisang lilin'	AA	$2n = 22$	X			
'Tjau lagada'	AA	$2n = 22$	X			
'Giant Cavendish'	AAA	$2n = 33$	X			
'Gros Michel'	AAA	$2n = 33$	X			
'Valery'	AAA	$2n = 33$	X			
'Yangambi Km5'	AAA	$2n = 33$	X			

*X indicates presence of diagnostic RAPD band.

Table 2. Genome composition of *Musa* hybrids determined by RAPD markers.

Acc. No.	Origin of hybrid	Ploidy	Diagnostic RAPD bands				Genome
			A17/D10	$A18_{200}$	$A18_{250}$	$A18_{300}$	
2829-62	BT (AAB) × C4 (AA)	$2n = 22$	X				AA
4400-8	BT × C4	$2n = 22$	X				AA
1448-1	OL (AAB) × C4 (AA)	$2n = 22$	X				AA
1297-3	FR (AAB) × C4 (AA)	$2n = 22$	X				AA
5105-1	PL (AA) × C4 (AA)	$2n = 22$	X				AA
9128-3	PL (AA) × TL (AA)	$2n = 22$	X				AA
15108-1	(BT × C4) × SH3362 (2*x*)	$2n = 33$	X	X	X		AAB
2796-5	BT × C4	$2n = 44$	X	X	X		AAAB
6930-1	OL × C4	$2n = 44$	X	X	X		AAAB
4698-1	OL × C4	$2n = 44$	X	X	X		AAAB
7152-2	ME (AAB) × C4	$2n = 44$	X	X	X		AAAB

Note: OL, 'Obina l'Ewai'; FR, 'French Reversion' ('Agbagba'); C4, 'Calcutta 4'; BT, 'Bobby tannap'; PL, 'Pisang lilin'; ME, 'Mbi Egome'; TL, 'Tjau lagada'.
*X indicates presence of diagnostic RAPD band.

erance, greater disease resistance, improved nutritional value, and increased starchiness were contributed by the B genome of *M. balbisiana* (Robinson 1996). Consequently, knowledge of the exact genomic composition of a cultivar or clone is important for *Musa* breeding.

Simmonds and Shepherd (1955) devised a system of classifying *Musa* into genomic groups based on scores for a range of morphological features. A plant's morphology can be altered by environmental factors, and such a system of morphological classification would be inconsistent. Addi-

Fig. 1. Species-specific RAPD patterns in *Musa acuminata* (lanes 1 and 2, 5 and 6, 9 and 10) and *M. balbisiana* (lanes 3 and 4, 7 and 8, 11 and 12) with primers A17, A18, and D10. Unique bands for A17, A18, and D10 are indicated with a dot (•), open circles (○), and an arrow head (▲), respectively. Lane M represents molecular weight markers in bp.

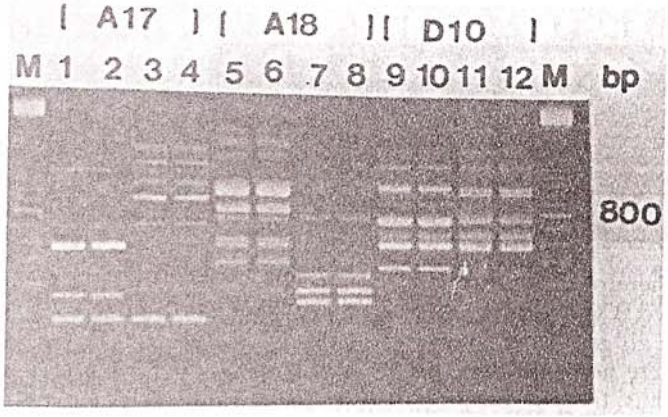
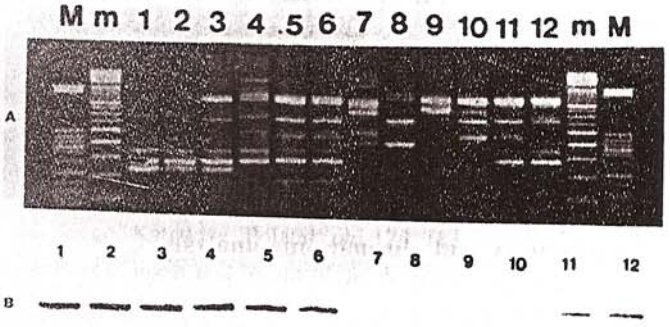


Fig. 2. (A) RAPD patterns of 12 *Musa* landraces with primer A18. Lanes 1–3 represent DNA from clones with two B genomes, lanes 4–6, 11, and 12 from clones with one B genome. Lanes 7–10 represent DNAs with no B genomes. (B) Southern hybridization pattern of A when fragment $A18_{250}$ (arrowhead) was used as a probe. The fragment does not hybridize to lanes 7–10 that contain only the A genomes.



tionally, some characteristics are only expressed at maturity and these traits can only be measured after 18–24 months in field plantations. Therefore, easier, more stable, and more reliable techniques are needed to determine genomic groups in *Musa*. Lysak et al. (1998) used flow cytometric analysis to predict genome composition in a few *Musa* genotypes. Although preliminary results showed a good correlation between nuclear DNA content and genome composition of the samples, an unusual clustering of AAB and AAA clones was observed (Lysak et al. 1998). Osuji et al. (1997) used genomic in situ hybridization (GISH) to identify the genome compositions of bananas, plantains, and artificial hybrids. Substantial cross-hybridization was reported between A and B genomes, making it difficult to unambiguously identify the origin of each chromosome. The GISH technique is a complex process requiring well-spread chromosomes on a slide, preparation of labeled probe DNA, denaturation of probe and chromosome spreads, hybridization, the removal by washing of unbound probe, and finally, the detection of hybridization sites (Trask 1991). Simpler techniques to identify genomes of a plant, preferably at the nursery stage, will generally have more appeal to plant scientists. Using 13 primers and a small sample of nine genotypes, Howell et al. (1994) identified RAPD (random amplified polymorphic DNA) markers that were specific to the nine genotypes of *Musa*, representing AA, AAA, AAB, ABB, and BB genomes. However, cluster analysis showed grouping of AAA with AAB clones, suggesting that these RAPD primers were only specific to the A genome.

This study aimed to assess the usefulness of RAPD markers for the identification of A and B genomes in *Musa*. This would provide a more rapid method to accurately determine a plant's genomic status, especially in breeding programs that involve interploidy crosses. Selection of plants on the basis of genomic composition could be done at the nursery stage from a few grams of leaf tissue, overcoming problems in *Musa* breeding that include the slow propagation of the plant, a very long life cycle (18–24 months), and the large space required for field testing (6 m^2 per plant). This study provides three RAPD markers that can be used to identify the genome composition of plantains and bananas.

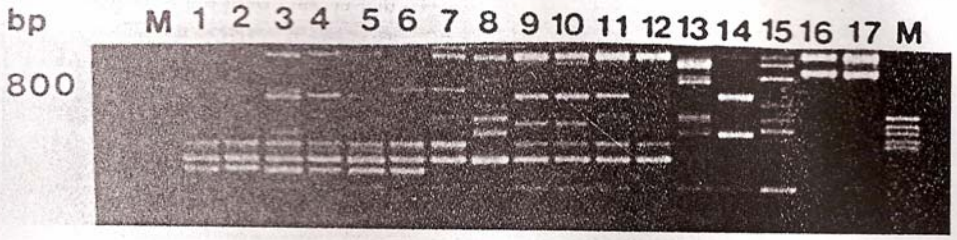
Materials and methods

The plants used in this study are listed in Tables 1 and 2. Table 1 contains landraces with known genomic compositions that were deduced from morphology. These plants were selected to represent a wide range of genomic groups. Table 2 contains diploid and tetraploid hybrid genotypes from the IITA breeding program. Genomic DNA was extracted from the samples according to the procedure described by Crouch et al. (1998). The DNA samples were quantified in a GeneQuant II RNA/DNA calculator (Pharmacia Biotech, U.K.). Reaction mixtures for RAPD analysis consisted of 0.2 µg DNA, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 1.25 U Taq Polymerase (Advanced Biotechnologies, Surrey, U.K.) and 1.2 µM of primer in a reaction buffer containing 75 mM Tris–HCl pH 9.0, and 20 mM $(NH_4)_2SO_4$ in a total volume of 25 µL. Eighty 10-mer primers from kits A, B, C, and D (Operon Technologies, Alameda, Calif.) were tested. Amplifications were carried out in a Perkin–Elmer Cetus 9600 Thermal Cycler with the following amplification conditions: an initial 3-min denaturation at 94°C followed by 35 cycles of 50 s at 94°C, 50 s 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 products was separated on 1.2% agarose gels in 1× TBE buffer. Molecular weight markers included the 100-bp ladder (Life Technologies, U.K.) and pBR322 fragments (Sigma, U.K.). The gels were stained in ethidium bromide and photographed under UV light.

Hybridization analysis

Amplified DNA fragments were transferred to nylon membranes as described by Reed and Mann (1985). The unique fragments were excised from gels and the DNA was cleaned from the gel slices according to Sambrook et al. (1989). These fragments were labeled with digoxigenin-11-dUTP using the Random Primer DNA Labeling Kit (Boehringer Mannheim). Detection of hybridization was performed with an alkaline phosphatase DIG Detection Kit (Boehringer Mannheim).

Fig. 3. PCR patterns of 17 *Musa* landraces with primer A18 showing bands unique to the B-genome clones. The lanes represent DNA from (1) *M. balbisiana* Hnd (BB), (2) *M. balbisiana* (Butohan 1) (BB), (3) 'Monthan Saba' (ABB), (4) 'Bluggoe' (ABB), (5) 'Cardaba' (ABB), (6) 'Klue Tiparot' (ABB), (7) 'Kisube' (AB), (8) 'Kamaramasenge' (AB), (9) 'Bobby tannap' (AAB), (10) 'Obino l'Ewai' (AAB), (11) 'Ngern' (AAAB), (12) 'Oura da mate' (AAAB), (13) *M. acuminata* 'Calcutta 4' (AA), (14) 'Pisang lilin' (AA), (15) 'Tjau lagada' (AA), (16) 'Giant Cavendish' (AAA), and (17) 'Valery' (AAA). Note that the three fragments (lane 1) characteristic of the B-genome clones are not present in A-genome clones (lanes 13–17). Lane M represents molecular markers in bp.



Flow cytometry and chromosome analysis

To release plant nuclei, about 20 mg of leaf tissue was chopped with a sharp razor blade in a glass petri dish containing 0.5 mL cold Otto I buffer (0.5 M citric acid monohydrate, 0.5% Tween–20). A further 0.5 mL of the Otto I buffer was added and the suspension was mixed with a pipette. The suspension was filtered through a 50-µm nylon mesh and kept at room temperature. To stain the DNA, 2 mL Otto II buffer (0.4 M anhydrous Na_2HPO_4) containing 4 µg/mL DAPI (4′,6-diamidino-2-phenylindole) was added. Fluorescence of DAPI-stained nuclei was analyzed using a Partec Ploidy Analyser (Partec GmbH, Germany). The gain of the instrument was set at channel 50 by using 'Calcutta 4' as a diploid standard. In this case, a triploid and tetraploid peak fall on channels 75 and 100, respectively. Slides for chromosome counting were prepared according to a protoplast releasing technique (Pillay et al. 2000) and stained with Leishman's stain.

Results and discussion

Landraces

Eighty 10-mer primers were used to amplify DNA from *M. acuminata* spp. *burmannicoides* clone 'Calcutta 4' (AA genomes) and *M. balbisiana* clone 'Honduras' (BB genomes). Three primers that produced unique clear bands for the differentiation of the two species were identified (Fig. 1). Primer A18 amplified three sequences, designated $A18_{200}$, $A18_{250}$, and $A18_{300}$, according to their molecular size (bp), in *M. balbisiana*. Two primers, A17 and D10, produced fragments of 600 bp and 320 bp, respectively, only in *M. acuminata*. To verify that the informative primers were specific to the A and B genomes, a sample of 29 genotypes representing 17 diploids, 10 triploids, and 2 tetraploids with various genome compositions (Table 1) were tested with these primers.

Fragments $A18_{250}$ and $A18_{300}$ were present in the *M. balbisiana* clones 'Monthan Saba', cooking bananas ('Bluggoe' and 'Cardaba'), 'Klue Tiparot', AB clones ('Kisube' and 'Kamaramasenge'), plantains ('Bobby tannap' and 'Obino l'Ewai'), and tetraploid genotypes ('Ngern' and 'Oura da mate') (Fig. 2). The $A18_{200}$ fragment was detected in all *M. balbisiana* clones, 'Monthan Saba', cooking bananas, and 'Klue Tiparot'. The A18 fragments were absent in A-genome diploid and triploid clones (Table 1). Fragments $A18_{250}$ and $A18_{300}$ were always present in genotypes containing at least one B genome, whereas the $A18_{200}$ fragment was present in clones only containing two B genomes. Therefore, fragment $A18_{200}$ is diagnostic for genotypes with two B genomes. Fragments $A17_{600}$ and $D10_{320}$ were detected in all genotypes that contained at least one A genome. They were not present in any of diploid BB genotypes (Table 1). Thus, these primers made it possible to elucidate the genome composition of all the plants in this study. For example, amplification of DNA from the AB clones with primers A18 and A17 showed the presence of an A-genome-specific fragment and fragments $A18_{250}$ and $A18_{300}$, which are characteristic for one B genome. The genomic composition of the clones 'Monthan Saba' and 'Bluggoe' were previously classified as BBB by Vakili (1967) while Valmayor et al. (1981) described 'Monthan Saba' as BBB and 'Bluggoe' as ABB. Our marker system showed that 'Monthan Saba' contained an A-specific fragment and all three A18 fragments, suggesting that it contained two B genomes. Therefore, 'Monthan Saba' should be placed into the ABB group in accordance with our data. Similarly, our results showed that genomic composition of 'Bluggoe' is ABB. 'Klue Tiparot', originally regarded as a tetraploid (ABBB) and reclassified as a triploid (Jenny et al. 1997; Horry et al. 1998), appeared as an ABB clone with our marker system. Our results show that classification of *Musa* germplasm into genomic groups primarily on the basis of phenotypic descriptors can be misleading.

Genome constitution of *Musa* hybrids

Genetic enhancement of *Musa* is based on crossing triploid AAB, ABB, or AAA landraces to diploid AA or BB accessions (Buddenhagen 1997). The triploid plantain, for example, can produce an array of gametes with genotypes (A), (AA), (AB), (B), and even an unreduced gamete (AAB). In wild diploid males, the production of reduced (A, B) and unreduced (AA, BB) gametes is determined by genotype (Ortiz 1997). For example, $2n$ gametes have not been observed in 'Calcutta 4', a male parent commonly used in breeding programs (Rowe and Rosales 1996). Therefore, diploid hybrids from a plantain × 'Calcutta 4' cross would have a genome composition of either AA or AB, triploids could be AAA or AAB, whereas tetraploids should be AAAB. Similarly, crosses between ABB landraces and an AA diploid could produce hybrids that are AA, AB, AAB, ABB, or AABB. In this study, we confirmed the ploidy level of the hybrids by flow cytometry and conventional chromosome counting, and determined their genome constitution (Ta-

ble 2) using our RAPD marker system. This method can be performed on very small plants that can be maintained in the greenhouse, and provides the *Musa* breeder with basic information about the hybrids.

This study identified PCR markers that are specific to the A and B genomes in *Musa*. Markers for the B genome can be used to distinguish cultivars containing a single B genome, such as in AB and AAB from those with two B genomes, such as BB and ABB. RAPD analysis provides a quick and reliable system for genome identification in *Musa* that could facilitate genome characterization and manipulation in breeding lines. The RAPD markers are being converted to sequence tagged sites (STSs) for greater reliability as a diagnostic tool. The STS assays are considered easier and less sensitive to variations in PCR conditions and DNA quality compared to RAPD analysis (Hu et al. 1997). We envision that our marker system will be useful in clarifying genome designations in the other sections of the genus *Musa*, namely *callimusa, australimusa*, and *rhodochlamys*. Our preliminary investigations in this regard show that *M. peekeli* (sect. *australimusa*) did not show the A- and B-genome-specific fragments after amplification with primers A17, A18, and D10. The section *australimusa* is considered to have the T genome (D'Hont et al. 1999).

Acknowledgements

We thank Dr. J. Dolezel, Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Olomouc, Czech Republic for setting up our Ploidy Analyzer, and C. Dimpka for excellent technical assistance. This research was supported, in part, by funds from the Directorate General for International Cooperation (DGIC ex-BADC), Belgium.

References

Buddenhagen, I.W. 1997. Overview of global banana breeding programs and research on diseases and pests of banana. *In* Banana Production, Biotechnology and Diversity. *Edited by* P. George, P. Stewart, and D.N. Vinh. Report of an International Workshop held at Hanoi, Vietnam, 9–11 October 1995. The World Bank, Washington, D.C. pp. 1–11.

Crouch, H.K., Crouch, J.H., Jarret, R.L., Cregan, P.B., and Ortiz, R. 1998. Segregation of microsatellite loci from haploid and diploid gametes in *Musa*. Crop Sci. 38: 211–217.

D'Hont, A., Paget-Goy, A., Jenny, C., Noyer, J.-L., Baurens, F.-C., Lagoda, P.J.L., and Carreel, F. 1999. Investigation of the complex genome structure of cultivated banana (*Musa* spp.) by flow cytometry, genomic DNA *in-situ* hybridization and repeated sequence analysis. *In* The International Symposium on the Molecular and Cellular Biology of Banana, held at Ithaca, New York, 22–25 March, 1999. *Edited by* P. Lagoda. INIBAP, Montpellier, France.

Horry, J.P., Dolezel, J., Dolezelova, M., and Lysak, M.A. 1998. Do natural A × B tetraploid bananas exist? INFOMUSA, **7**: 5–6.

Howell, E.C., Newbury, H.J., Swennen, R.L., Withers, L.A., and Ford-Lloyd, B.V. 1994. The use of RAPD for identifying and classifying *Musa* germplasm. Genome, **37**: 328–332.

Hu, X.Y., Ohm, H.W., and Dweikat, I. 1997. Identification of RAPD markers linked to the gene PM1 for resistance to powdery mildew in wheat. Theor. Appl. Genet. **94**: 832–840.

Jenny, C., Carreel, F., and Bakry, F. 1997. Revision on banana taxonomy: "Klue Tiparot" (*Musa* sp.) reclassified as a triploid. Fruits (Paris), **52**(2): 89–91.

Lysak, M., Dolezelova, M., and Dolezel, J. 1998. Flow cytometric analysis of nuclear genome size in *Musa* spp. *In* Plants Cytogenetics. *Edited by* J. Maluszynska. Wydawnictwo Uniwersytetu Slaskiego, Katowice, Poland. pp. 178–183.

Ortiz, R. 1997. Occurrence and inheritance of 2*n* pollen in *Musa*. Ann. Bot. **79**: 449–453.

Osuji, J.O., Harrison, G., Crouch, J., and Heslop-Harrison, J.S. 1997. Identification of the genome constitution of *Musa* L. lines (bananas, plantains and hybrids) using molecular cytogenetics. Ann. Bot. **80**: 789–793.

Pillay, M., Tenkouano, A., and Hartman, J. 2000. Future challenges in *Musa* breeding. *In* Crop Improvement for the 21st Century. *Edited by* M. Kang. Food Products Press, New York.

Reed, K.C., and Mann, D.A. 1985. Rapid transfer of DNA agarose gels to nylon membranes. Nucleic Acids Res. **13**: 7207–7221.

Robinson, J.C. 1996. Bananas and Plantains. CAB International, U.K.

Rowe, P., and Rosales, F.E. 1996. Bananas and plantains. *In* Fruit Breeding. Vol. I: Tree and Tropical Fruits. *Edited by* J. Janick and J.N. Moore. John Wiley and Sons Inc., New York. pp. 167–211.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Simmonds, N.W. 1995. Bananas *Musa* (Musaceae). *In* Evolution of Crop Plants. 2nd ed. *Edited by* J. Smartt and N.W. Simmonds. Longmans Scientific and Technical, Essex, U.K. pp. 370–375.

Simmonds, N.W., and Shepherd, K. 1955. The taxonomy and origins of the cultivated bananas. J. Linn. Soc. Lond. Bot. **55**: 302–312.

Stover, R.H., and Simmonds, N.W. 1987. Bananas. 3rd ed. Longman, London.

Trask, B.J. 1991. Fluorescence *in situ* hybridization: Applications in cytogenetics and gene mapping. Trends Genet. **7**: 149–154.

Vakili, N.G. 1967. The experimental formation of polyploidy and its effect in the genus *Musa*. Am. J. Bot. **54**: 24–36.

Valmayor, R.V., Rivera, F.N., and Lomuljo, F.M. 1981. Philippine banana cultivar names and synonyms. National plant genetic resources laboratory Bull. No. 3. Institute of plant breeding, University of Philippines, Los Banos.