Ploidy and Genome Segregation in *Musa* Breeding Populations Assessed by Flow Cytometry and Randomly Amplified Polymorphic DNA Markers

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ABSTRACT. The majority of edible bananas (*Musa* L.) are triploid ($2n = 3x = 33$) cultivars known as dessert, plantain, or cooking bananas with predominant AAA, AAB, and ABB genomes, justifying the aim of breeding programs to develop improved hybrids of the different utilization classes in the triploid background. This usually involves crossing $3x$ landraces to $2x$ accessions that are donors of resistance genes, selecting $4x$ and $2x$ primary hybrids from the $3x-2x$ progenies, and crossing $4x-2x$ hybrids to produce secondary $3x$ hybrids. Ploidy and genome segregation routinely occurs during this process and constitutes a major hindrance for selection because this is not easily detectable at the morphological level. This study was carried out to assess the potential of early screening for discriminating ploidy and genome classes in $4x$ (AAB)– $2x$ (AA) breeding populations, based on flow cytometry and genome-specific RAPD analysis. Results indicate that progenies of $4x-2x$ crosses produced predominantly $3x$ progenies (94.1%). RAPD analysis identified seven genomic classes, dominated by AAA and AAB configurations segregating 1:1 ($\chi^2 = 0.21$, $P = 0.05$). Plant size and bunch weight increased with ploidy level, justifying ploidy-based grouping of progenies for homogenous field evaluation. Similarly, genome-based grouping within ploidy classes is recommended to allow proper evaluation of progenies according to targeted postharvest utilization. Thus, ploidy analysis by flow cytometry and genome determination by RAPD open prospects for enhancing breeding efficiency in the development of triploid bananas or plantains.

Banana and plantain are important cash and subsistence crops in most tropical and subtropical regions of the world (Robinson, 1996). The cultivars that are used for commercial or subsistence production are predominantly triploid ($2n = 3x = 33$) cultivars that are evolutionary derived from crosses within and between diverse accessions of two diploid ancestor species, *Musa acuminata* Colla (A genome) and *M. balbisiana* Colla (B genome).

Ploidy and genome configurations have played a determinant role in the classification of bananas, with major groups including diploids (AA, BB, AB), triploids (AAA, AAB, ABB) and tetraploids (AAAA, AAAB, AABB, AB BB). Clones are assigned to genomic groups on the basis of morphological characters. In this classification, subgroups are made of clones that have similar characteristics, having arisen from a single base clone by somatic mutation.

The existing cultivars are usually classified into three genome groups, AAA, AAB, and ABB but the cultivars grouped in the same genomic category can be very different (Robinson, 1996; Simmonds, 1962; Stover and Simmonds, 1987). Thus, the AAA group consists of both sweet dessert bananas that are eaten raw when ripe and bananas of the east African highlands that require cooking before they can be eaten or brewing before drinking.

Likewise, the west African and Asia Pacific (Maia maoli/Popoulou) plantains that are cooked before eating dominate the AAB group, which also contains dessert bananas of the Pome subgroup. In contrast, cultivars in the ABB group are predominantly used for cooking although some may be eaten raw as dessert bananas usually when overripe. While various combinations of the A and B genomes predominate in most cultivars, two other genomes, the S genome (*M. schizocarpa* Simmonds) and T genome (*M. textilis* Nee), occur albeit in a few accessions (Carreel, 1995; Sharrock, 1990; Tezenas du Montcel, 1990).

There is wide consensus about the attributes conferred by A or B genomes in interspecific natural or artificial hybrids of *M. acuminata* and *M. balbisiana*. Hence it is accepted that edibility of mature fruits arose from mutations causing parthenocarpy and female sterility in diploid *M. acuminata* (Simmonds, 1962). It is also commonly accepted that hardiness is contributed by the B genome since *M. balbisiana* clones thrive abundantly in areas experiencing pronounced dry seasons alternating with monsoons. Also attributed to the B genome are fruit characteristics such as starchiness and acid taste, causing AAB plantain to be starchier but less sweet and less palatable when raw than the AAA dessert bananas (Simmonds, 1962).

Because of biotic stresses such as black sigatoka (caused by *Mycosphaerella fijiensis* Morelet), considerable efforts have been made to breed for resistance, as well as to broaden the genetic base of these crops (Vuylsteke et al., 1997). The aim is to produce seedless cultivars, preferably in the triploid background that is common to the majority of existing cultivars. This usually involves crossing $3x$ cultivars to $2x$ accessions that are donors of resistance genes, selecting $4x$ and $2x$ primary hybrids from the $3x-2x$ progenies, and crossing $4x-2x$ hybrids to produce secondary $3x$ hybrids. Alternatively, secondary $3x$ hybrids may be produced via $2x-2x$ crosses where one of the $2x$ parents produces

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2n pollen. However, this requires screening of diploid lines for 2n pollen production, and setting crossing schemes that would allow pollination to coincide with periods that are favorable for 2n pollen production.

Other methods of producing triploid hybrids have been discussed. One such method involves tetraploidization of diploid accessions using colchicine prior to crossing with another diploid, which has been advocated by Novak (1992) and Tezenas du Montcel et al. (1996). Colchicine prevents the formation of mitotic spindles, which essentially results in mitotic restitution in treated cells. An attractive feature of this technique is the preservation of advantageous linkages selected at diploid level, particularly in the tetraploid derived from the diploid. However, recombination can occur when crossing the tetraploid with a diploid to produce a triploid, with the potential of disrupting linkages. Also, colchicine treatment may not affect uniformly all cells in multicellular meristems, causing cytochimeras that may not be easy to dissociate (Roux et al., 2001). Thus, efficient methods for in vitro dissociation of chimeras and selection of the desired cells are required, before such cells can be cultured to regenerate a plant that will now be used for cross-breeding. Furthermore, the use of colchicine could result in increased inbreeding, reduced vigor and reduced genetic variability (Ortiz et al., 1992).

Hence, tetraploid x diploid crosses remain the predominant triploid breeding scheme, although the multiploidy and heterogenic structure of breeding populations results in unpredictable variation in genome size and structure across and within generations. This causes complex inheritance patterns and complicates phenotypic selection for most yield and growth traits (Ortiz and Vuylsteke, 1996).

The objective of this study was to analyze the distribution patterns of ploidy and genome configurations in tetraploid x diploid breeding populations of plantain and banana.

Materials and Methods

PLANT MATERIALS. An experimental population was generated by crossing five tetraploid AAAB accessions (OSH20, OSH27, OSH35, OSH45, OSH46) to five diploid AA accessions (OSH38, OSH49, OSH52, OSH60 and OSH63). Pedigree details of the parental accessions are given in Table 1. Only 13 of the 25 cross combinations produced viable seeds, from which zygotic embryos were extracted and germinated in vitro (Vuylsteke et al., 1990). The resulting seedlings were nursery hardened in polyethylene bags containing a mixture of topsoil, chicken manure and coconut fibre in 7:1:2 ratio. Parental genotypes were also clonally propagated using meristematic tissue from shoot tips (Vuylsteke et al., 1990).

FIELD EXPERIMENT. Approximately 2-month-old seedlings were transplanted on 6 June 1999 in the field along with the parental accessions at the High Rainfall Station of the International Institute of Tropical Agriculture (IITA) at Onne (lat. 4°43'N, long. 7°01'E, 10 m above sea level) in southeastern Nigeria. The station is located in a degraded rainforest-swamp characterized by an Ultisol/Acrisol [U.S. Department of Agriculture (USDA) Taxonomy/World Reference Base (WRB)] derived from coastal sediments and 2400 mm unimodal annual rainfall. The soil is a deep and freely drained Typic Paleudult/Haplic Acrisol of the coarse-loamy, siliceous isohyperthermic family, with poor nutrient status and low pH (pH 4.3 in 1:1 H₂O in the upper 15 cm). Detailed characteristics of the Onne station have been described elsewhere (Ortiz et al., 1997).

The experimental layout was an unbalanced randomized complete-block design with two replications, whereby progenies from the different families were arbitrarily distributed to one or the other replication. Parental clones were represented by a minimum of five plants in each replication. Standard controls used in all our polyploid breeding trials included 'Agbagba' (most preferred landrace in Nigeria), 'PITA16' (secondary triploid hybrid), and 'PITA14' (tetraploid hybrid currently disseminated in Nigeria), also represented by a minimum of five plants in each replication. Plant spacing was 3 m between rows and 2 m within rows to give a population density of 1667 plants per hectare. The plants were grown for two consecutive crop cycles (one cycle = approximately 1 year) according to the specifications of Swennen (1990) and data were recorded on several phenological and yield traits.

PLOIDY ANALYSIS. Leaf samples were collected from the cigar (emerging tightly rolled), or youngest fully expanded, leaf of field-grown plants and immediately stored in ice packs. About 50 mg of mid-rib tissue was chopped with a sharp razor blade in a petri dish with 0.5 mL ice-cold Otto I buffer (0.5 M citric acid monohydrate, 0.5% Tween 20) to release cell nuclei. Another 0.5 mL Otto I buffer was added to the suspension, which was filtered through a 50-µm nylon mesh and kept at room temperature. The suspension of released cell nuclei was stained by addition of 2 mL Otto II buffer (0.4 M anhydrous Na₂HPO₄) containing 4 µg·mL⁻¹ DAPI (4,6-diamidino-2-phenylindole).

Fluorescence detection was carried out with a Partec Ploidy Analyzer PA-II (Partec GmbH, Münster, Germany) whereby relative fluorescence intensities were translated into histograms corresponding to the relative DNA content, hence ploidy status, of tested samples (Dolezel, 1997). Two reference accessions of

Table 1. Pedigree details of five tetraploid (4x) and five diploid (2x) *Musa* lines used to develop 4x-2x families segregating for ploidy and genome configurations.

Selection no. ^z	Breeding no. ^y	Pedigree ^x
Females (4x)		
OSH20	TMPx1658-4	'Obino l'Ewai' x 'Pisang Lilin'
OSH27	TMPx2796-5	'Bobby Tannap' x 'Pisang Lilin'
OSH35	TMPx4698-1	'Obino l'Ewai' x 'Calcutta 4'
OSH45	TMPx6930-1	'Obino l'Ewai' x 'Calcutta 4'
OSH46	TMPx7002-1	'Obino l'Ewai' x 'Calcutta 4'
Males (2x)		
OSH38	TMB2x5105-1	'Pisang lilin' x 'Calcutta 4'
OSH49	TMB2x7197-2	SH3362 x 'Long Tavoy'
OSH52	TMB2x8084-2	SH3362 x 'Calcutta 4'
OSH60	TMB2x9719-7	'Manang' x 'Calcutta 4'
OSH63	TMB2x9839-2	'Calcutta 4' x 'Padri'

^zThe International Institute of Tropical Agriculture operates two breeding programs, one for the humid lowlands of western and central Africa and another for the mid-altitude and highlands of eastern and southern Africa, respectively based at Onne (Nigeria) and Namulonge (Uganda), hence the OSH designation for Onne-selected hybrids and NSH for Namulonge-selected hybrids.

^ySerial cross number with prefix TMP or TMB standing for "tropical *Musa* plantain" (plantain-derived hybrid) or "tropical *Musa* banana" (banana-derived hybrid).

^xThe accessions 'Bobby Tannap' and 'Obino l'Ewai' are triploid AAB plantains from west Africa that are susceptible to black sigatoka; 'Calcutta 4', 'Long Tavoy', 'Manang', 'Padri', and 'Pisang Lilin' are diploid *Musa acuminata* (AA) accessions from southeast Asia that are resistant; SH3362 is a bred diploid hybrid from the Fundación Hondureña de Investigación Agrícola (FHIA) in Honduras.

known ploidy level, 'Calcutta 4' (diploid) and 'Obino l'Ewai' (triploid), were used as internal standards and the analytical instrument was calibrated so that the G_1 peak of nuclei isolated from the control diploid plant was on channel 50, while that of the triploid was on channel 75 (Pillay et al., 2000). This setting was kept constant during analysis of samples prepared from the breeding population to compare their peak or histogram to that of the reference plants. Thus, peaks appearing on channels 50, 75 and 100 corresponded to diploid, triploid, and tetraploid plants, respectively (Fig. 1).

Peak records were used to construct the frequency distribution of ploidy classes for each $4x-2x$ family. Field-grown progenies were assumed to be euploid individuals arising from the fusion of euploid gametes of $4x$ or $2x$ origin. This is because seedlings from aneuploid progenies seldom survive to field establishment and most of those that do survive usually show very poor growth and seldom reach maturity. Euploid gametes from $4x$ origin would be $1x$, $2x$, $3x$, or $4x$ while those of $2x$ origin would be $1x$ and $2x$, giving five euploid sporophytic classes (i.e., $2x$, $3x$, $4x$, $5x$, and $6x$). Chi-square analysis was used to test for nonpreferential occurrence of a particular sporophytic ploidy class in the $4x-2x$ families.

Flow cytometry is one of three methods used for ploidy determination, the other two consisting of chloroplast counts in stomata guard cells (Tenkouano et al., 1998) and, when in doubt, chromosome counts (Pillay and Adeleke, 2001).

GENOME ANALYSIS. DNA was extracted from about 450 plants of the $4x-2x$ families, the parental accessions, and two reference homogenomic diploid *M. acuminata* ('Calcutta 4') and *M. balbisiana* ('*M. balbisiana* Los Banos') clones. Cigar leaf samples were collected and immediately immersed in liquid nitrogen, prior to grinding. The samples were ground in liquid nitrogen using a pyrex mortar and pestle and the ground tissue was transferred into Eppendorf tubes (Eppendorf, Hamburg, Germany) containing preheated extraction buffer [4% hexadecyltrimethylammonium bromide (CTAB), 100 mM Tris-HCl (pH 8.00), 1.4 M NaCl, 20 mM ethylenediaminetetraacetic acid (EDTA), 4 $\mu\text{L}\cdot\text{mL}^{-1}$ β -mercaptoethanol] and incubated in a water bath (65 °C) following the procedure of Crouch et al. (1998) and Gawal and Jarret (1991).

DNA was extracted by addition of chloroform: isoamyl alcohol (24:1 v/v) followed by centrifugation at 725 g_n for 3 min to remove proteins and precipitation of the supernatant with two volumes of ice-cold isopropanol. DNA was recovered by centrifuging at 2900 g_n for 5 min and the DNA pellets were suspended in TE buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0). RNA contamination was removed by digestion with 10 $\mu\text{g}\cdot\text{mL}^{-1}$ RNase for 30 min at room temperature. Finally, stock DNA was adjusted to 40 $\text{ng}\cdot\mu\text{L}^{-1}$ in TE buffer and kept in a refrigerator (4 °C) for use in subsequent assays.

Amplification by polymerase chain reaction (PCR) was carried out with 25- μL reaction mixtures containing 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 buffer containing 75 mM Tris-HCl pH 9.0, and 20 mM $(\text{NH}_4)_2\text{SO}_4$. Primers A17 and A18 with respective sequences of GACCGCTTGT and AGGTGACCGT (Operon Technologies, Alameda, Calif.) were used for genome determination as previously described (Pillay et al., 2000).

Amplification was carried out in a Perkin-Elmer Cetus 9600 Thermal Cycler (Perkin-Elmer Life and Analytical Sciences, Inc., Wellesley, Mass.) with 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 10 μL of the amplification products was separated on 1.2% agarose gel in 1x TBE buffer. Molecular weight markers included the 100-bp ladder (Invitrogen Life Technologies Ltd., Paisley, U.K.) and pBR322 fragments (Sigma-Aldrich Company Ltd., Dorset, U.K.). The gels were stained in ethidium bromide and visualized under ultraviolet light.

Polymorphic bands distinguishing the A and B genomes were scored in all the samples and genomic classification was made in conjunction with ploidy status as previously described (Pillay et al., 2000).

AGRONOMIC EVALUATION. In order to illustrate differences in performance that could be attributed to variation in ploidy and genome configuration, individuals were grouped according to such configurations, and statistical analysis was carried out to assess differences among groups for bunch weight, plant height, and plant girth, using the *t* test (Singh and Chaudhary, 1985), whereby:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S^2_{x_1}}{n_1} + \frac{S^2_{x_2}}{n_2}}}$$

where "t" is the observed test statistic for the difference between means of two ploidy groups; $\bar{X}_1$, $\bar{X}_2$ represent the means of ploidy groups X_1 , X_2 , respectively, for a particular trait; $S^2_{x_1}$, $S^2_{x_2}$ represent the associated variances, and n_1 , n_2 represent the number of individuals of the ploidy groups. The calculated value of "t" was compared with the tabulated value of "t" for significance at $n_1 + n_2 - 2\text{df}$.

Results and Discussion

PLOIDY ANALYSIS. Significant differences in ploidy class distribution were found for all the $4x-2x$ families, with a preponderance of $3x$ progenies (Table 2). It thus appears that $4x-2x$ crosses predominantly produce $3x$ progenies, which is expected under the assumption of normal meiosis in both parents. Hence, despite the multiple ploidy and heterogenomic nature of the breeding lines used as parents, this study shows that the $4x-2x$ breeding scheme constitutes a reliable means of generating triploid hybrids. The relative frequency of such event was higher than 90% in all $4x-2x$ families, except one: OSH27 x OSH52 with 84% triploids and 16% diploids. Double reduction resulting in the production of hypoploid gametes ($1x$) was observed to occur during microsporogenesis in $4x$ hybrids (unpublished data). Whether double reduction also occurs during megasporogenesis has not been experimentally verified, but this would tentatively explain the origin of the $2x$ progenies in $4x-2x$ crosses, particularly in the lineage of 'Bobby Tannap', an accession that has consistently produced more diploid than polyploid hybrids compared to other landraces (Vuylsteke, 2001).

Hyperploidies were only observed in five families, indicating that such families may require greater preplanting scrutiny to avoid field evaluation of potentially aberrant phenotypes associated with increased genome size. Conversely, hypoploid progenies were observed in all but three families (OSH46 x OSH49, OSH46 x OSH52, and OSH46 x OSH60), indicating that these families may not be suitable for diploid breeding aimed at producing improved genetic stocks intended for use as male parents (Tezenas du Montcel et al., 1996).

Fig. 1. Typical histograms of 4-6-diamidino-2-phenylindole (DAPI)-labeled cell nuclei at pre-DNA synthesis phase (G1) from (A) a diploid, (B) a triploid, and (C) a tetraploid *Musa* sp. plant subjected to flow cytometry. FL1 indicates the fluorescence signal intensity, which stoichiometrically relates to DNA content.

Table 2. Frequency distribution and chi-square test of equal segregation of ploidy configurations in 13 *Musa* families derived from 4x–2x crosses. Ploidy determination by flow cytometry of field-grown plants was carried out at the high rainfall station of the International Institute of Tropical Agriculture (IITA) at Onne, southeastern Nigeria.

Family	N ^z	Ploidy class				χ^2 value
		2x	3x	4x	5x	
		-----%-----				
OSH20 x OSH38	45	4.4	93.3	2.2	0.0	112.60**
OSH20 x OSH49	55	1.8	92.7	5.5	0.0	134.96**
OSH20 x OSH52	28	7.1	92.9	0.0	0.0	69.71**
OSH20 x OSH63	72	9.7	90.3	0.0	0.0	168.17**
OSH27 x OSH52	75	16.0	84.0	0.0	0.0	152.04**
OSH27 x OSH60	30	3.3	96.7	0.0	0.0	82.40**
OSH27 x OSH63	82	3.7	90.2	4.9	1.2	186.83**
OSH35 x OSH52	53	3.8	96.2	0.0	0.0	143.91**
OSH45 x OSH49	48	4.2	95.8	0.0	0.0	129.00**
OSH45 x OSH63	29	3.4	96.6	0.0	0.0	79.41**
OSH46 x OSH49	15	0.0	93.3	6.7	0.0	37.53**
OSH46 x OSH52	27	0.0	96.3	3.7	0.0	73.30**
OSH46 x OSH60	17	0.0	100.0	0.0	0.0	51.00**

^zNumber of informative individuals.

**Indicates significant departure from the assumption of equal class distribution at the 0.01 probability level.

Table 4. Chi-square test for equal distribution of genome configurations in triploid (3x) progenies of 13 *Musa* families derived from crosses between tetraploid (4x) and diploid (2x) lines with AAAB and AA genomes, respectively. Genome determination by RAPD was carried out for field-grown plants at the high rainfall station of the International Institute of Tropical Agriculture (IITA) at Onne, southeastern Nigeria.

Family (AAAB x AA)	Genome configurations in 3x progeny		
	AA.A	AB.A	$\chi^2_{0.05}$ (1 df)
OSH20 x OSH38	22	16	0.66
OSH20 x OSH49	19	30	2.04
OSH20 x OSH52	11	12	0.00
OSH20 x OSH63	8	5	0.31
OSH27 x OSH63	34	32	0.02
OSH27 x OSH60	8	9	0.00
OSH27 x OSH63	22	18	0.23
OSH31 x OSH52	11	5	1.56
OSH45 x OSH49	26	25	0.00
OSH45 x OSH63	15	17	0.03
OSH46 x OSH49	9	5	0.64
OSH46 x OSH52	7	20	5.33 *
OSH46 x OSH60	3	11	3.57
Overall	195	205	0.21
Pooled $\chi^2_{0.05}$ (13 df)	---	---	14.39
Heterogeneity $\chi^2_{0.05}$ (12 df)	---	---	14.18

*Indicates significant departure from equal distribution at 0.05 probability level.

Table 3. Genome configurations in 13 *Musa* families derived from crosses between tetraploid and diploid lines with AAAB and AA genomes, respectively. Genome determination by RAPD was carried out for field-grown plants at the high rainfall station of the International Institute of Tropical Agriculture (IITA) at Onne, southeastern Nigeria.

Family (AAAB x AA)	N ^z	Genome configurations						
		AA	AB	AAA	AAB	AAAA	AAAB	AAAAB
		-----%-----						
OSH20 x OSH38	41	2.4	2.4	53.7	39.0	0.0	2.4	0.0
OSH20 x OSH49	54	1.9	1.9	35.2	55.6	1.9	3.7	0.0
OSH20 x OSH52	25	8.0	0.0	44.0	48.0	0.0	0.0	0.0
OSH20 x OSH63	13	0.0	0.0	61.5	38.5	0.0	0.0	0.0
OSH27 x OSH52	69	0.0	4.3	49.3	46.4	0.0	0.0	0.0
OSH27 x OSH60	18	0.0	5.6	44.4	50.0	0.0	0.0	0.0
OSH27 x OSH63	44	0.0	0.0	50.0	40.9	0.0	6.8	2.3
OSH35 x OSH52	16	0.0	0.0	68.8	31.3	0.0	0.0	0.0
OSH45 x OSH49	53	0.0	3.8	49.1	47.2	0.0	0.0	0.0
OSH45 x OSH63	33	3.0	0.0	45.5	51.5	0.0	0.0	0.0
OSH46 x OSH49	14	0.0	0.0	64.3	35.7	0.0	0.0	0.0
OSH46 x OSH52	27	0.0	0.0	25.9	74.1	0.0	0.0	0.0
OSH46 x OSH60	14	0.0	0.0	21.4	78.6	0.0	0.0	0.0

^zNumber of informative individuals.

GENOME ANALYSIS. Discriminating genomes is of both practical and theoretical interest for *Musa* breeders, hence various methods to identify genomes in *Musa* have been reported. In this study, genome identification was carried out with RAPD markers linked to A and B genome sequences in *Musa* (Pillay et al., 2000). Scoring of polymorphic bands resulting from priming with A17 and A18 was done as described by Pillay et al. (2000). Parental genomes were AAAB for the tetraploids and AA for the diploids, whereas, the progenies displayed seven genomic configurations, namely, AA, AB, AAA, AAB, AAAA, AAAB, and AAAAB (Table 3). The triploid individuals segregated into two genome configurations, but one family, OSH46 x OSH52, statistically departed from the 1AAA: 1AAB ratio ($\chi^2 = 5.33$, P

< 0.05). The chi-square values for the other families and cross-family statistics were nonsignificant at the 0.05 probability level (Table 4).

Thus, triploid hybrids essentially segregated following a 1:1 ratio, which may indicate the lack of genome specificity in chromosome assortment in the AAAB female parent. This lends support to the views of Sathiamoorthy and Balamohan (1993) who suggested that *Musa* genomes might be highly homeologous. However, the results of Ude et al. (2002) suggested that there was sufficient genetic differentiation between and within the A and B genomes, in agreement with Lysak et al. (1999) who found differences in the size of the A and B genomes.

The RAPD markers are useful for initial determination of

genome composition of breeding populations and provide more objective means for classification than does the morphology-based approach of Simmonds and Shepherd (1955). This is because plant morphology depends on the environment, requiring evaluation over many locations and cropping cycles for reliable classification of hybrids. Furthermore, the determination cannot be completed if some characters cannot be scored (Perrier and Tezenas du Montcel, 1990).

However, RAPD fragments are anonymous sequences that may be prone to distorted segregation (Faure et al., 1993) and may not have enough discriminatory power (Howell et al., 1994). Furthermore, incomplete differentiation between A and B genomes suggests that the chromosomes belonging to either group might not behave as members of distinct clusters, which might be further complicated by probable recombination of A-specific and B-specific segments (Ortiz and Vuylsteke, 1994). Thus, other types of DNA markers have been sought for. In this regard, Nwakanma et al. (2003) used PCR-RFLP of the internal transcribed spacer regions of the ribosomal RNA genes to identify markers for the A and B genomes, whereby a 530-bp fragment unique to the A genome and two fragments of 350 bp and 180 bp specific for the B genome were identified. Interspecific cultivars with both A and B genomes had all three fragments with a dosage effect observed for the B genomes since the staining intensity of accessions with two B genomes was approximately two times that of accessions with a single B genome.

Ideally, molecular markers for genome composition of bananas should be specific for each of the 11 linkage groups present in the Eumusa series. In this regard, only methods using genomic in situ hybridization (GISH) could prove satisfactory, as demonstrated by D'Hont et al. (2000), Osuji (1997), and Osuji et al. (1998), but these techniques are not readily applicable to routine analysis of breeding populations. In addition, cross hybridization between genomes was reported by both D'Hont et al. (2000) and Osuji et al. (1998), reflecting the sequence homologies and affinities between the genomes, which was earlier advocated (Sathiamoorthy and Balamohan, 1993). Cross-hybridization intensity may be indicative of the genetic distances between cultivars representing the different genomes.

The reported GISH experiments produced broad signals across the centromeric regions of all the chromosomes with some chromosomes showing stronger hybridization signals than others, but the chromosomes were not uniformly stained across their length (D'Hont et al., 2000; Osuji et al., 1998). According to Pillay et al. (2004), the differential hybridization signals along the length of the chromosome may be due to the organization of the different classes of repetitive DNA. Thus, *Musa* chromosomes contract differentially during prometaphase and metaphase, and the centromeric regions appear to be more highly condensed than the distal regions suggesting that condensation of chromosomes in *Musa* begins in the centromeric region, the lagging distal regions often appearing as lightly stained tails (Pillay et al., 2004). Hence the GISH technique only identifies chromosome segments that have "A" or "B" origin.

Furthermore, the finding that the cultivar 'Pelipita' had eight A chromosomes and 25 B chromosomes instead of the 11 A and 22 B expected (Osuji et al., 1998) did not result in questioning its classification as an ABB accession. Similarly, the AAB plantain status of the cultivars 'Mboureoukou' and 'Nyombe' was not challenged by the discovery by D'Hont et al. (2000) that they had 21 A and 12 B chromosomes. Also, whether the apparently supernumerary "B chromosomes" were merely B-specific ma-

terials translocated to A-dominated chromosomes was not ruled out in either study.

Thus, no convenient quantitative method for determination of genome composition is currently available, and the classifications provided in this study are only indicative of the presence of A and B genome-specific materials, not necessarily as nonrecombined or complete sets of the A or B chromosomes. Nevertheless, this study shows that breeding populations can be ascribed to various ploidy and genome categories before field evaluation.

AGRONOMIC PERFORMANCE. As expected, bunch weight, plant height and pseudostem girth increased on the average with ploidy status (Table 5), similar to the findings of Ortiz (1997) and Tezenas du Montcel et al. (1996). This is probably as a result of higher order (i.e., tri-allelic and tetra-allelic, epistatic interactions in polyploid individuals) compared to diploid progenies (Peloquin and Ortiz, 1992). More specifically, pseudostem girth was significantly different among ploidy classes, whereas no differences were found for the plant height of diploid and triploid classes or the bunch weight of triploid and tetraploid groups (Table 5). Within the triploid class, the AAA or AAB sub-groups significantly differed for plant height but not for bunch weight and pseudostem girth (Table 5). Although the tetraploid hybrids produced good bunches, they were usually female and male fertile, which reduces fruit quality due to the presence of seeds in the pulp (Ortiz and Vuylsteke, 1996). In contrast, triploid hybrids have more propensity to be seedless, which constitutes an advantage over the tetraploid hybrids.

Conclusion

Ploidy analysis by flow cytometry and genome determination by RAPD open prospects for enhancing tetraploid x diploid breeding efficiency for the development of triploid hybrids. Thus, *Musa* breeders may incorporate ploidy and genome analysis at the nursery stage preceding field establishment of hybrids in order to select triploid breeding lines with putative plantain, dessert or cooking banana utilization classes.

Table 5. Field performance of diploid (2x), triploid (3x) and tetraploid (4x) *Musa* progenies from 4x-2x crosses for bunch weight, plant height and plant girth (50 cm above soil line) and *t* test of the difference between means. Ploidy and genome groupings were based on flow cytometry and RAPD analysis, respectively. Data were obtained for plants grown for two consecutive crop cycles (June 1999 to June 2001) at the high rainfall station of the International Institute of Tropical Agriculture (IITA) at Onne, southeastern Nigeria.

Ploidy	Plant characteristics		
	Bunch wt (kg)	Plant ht (cm)	Plant girth (cm)
	-----Means (SD) -----		
Diploids (2x)	1.9 (2.1)	256 (47)	39 (10)
Triploids (3x)	3.5 (3.4)	278 (61)	47 (10)
AAA	3.5 (3.5)	266 (60)	46 (10)
AAB	3.6 (3.3)	288 (60)	47 (10)
Tetraploids (4x)	5.1 (4.0)	321 (45)	53 (6)
	--- <i>t</i> test for difference between progeny means ---		
2x vs. 3x	**	NS	**
2x vs. 4x	*	**	***
3x vs. 4x	NS	**	**
Within 3x (AAA vs. AAB)	NS	*	NS

NS, *, **, ***Nonsignificant or significant differences between means at $P \leq 0.05$, 0.01, and 0.001, respectively.

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