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Full Length Research Paper

Ploidy and genome composition of *Musa* germplasm at the International Institute of Tropical Agriculture (IITA)

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***Musa* spp (bananas and plantains) constitute a hybrid-polyploid complex and are classified according to different genome compositions such as AA, BB, AB, AAA, AAB, ABB, AAAA, ABBB, AAAB and AABB. Knowledge of ploidy and exact genome compositions of the parental material is essential for *Musa* breeding. This study determined the ploidy levels and genome composition of the *Musa* germplasm collection, constituting over 300 accessions, at the International Institute of Tropical Agriculture in Nigeria and Uganda. Flow cytometric analysis of nuclear DNA content was used to estimate ploidy levels, while genome composition was ascertained with RAPD markers that are specific for the A and B genomes of *Musa*. It was determined that at least 8% of the plants in the germplasm collection were miss-classified in terms of ploidy and/or genome composition. The cultivars 'Pisang awak', 'Foulah 4' and 'Nzizi', previously classified as triploids, were found to be tetraploids by flow cytometry and conventional root tip chromosome counts. Similarly, cultivars that were previously classified as diploids including 'Too', and 'Toowoolee' were found to be triploids in our analysis. Ploidy and genome classification in *Musa* was generally determined from morphological characteristics. While our study showed that such a system is not always reliable, it was interesting to find that none of the plantains in the germplasm collection were miss-classified with regards to both ploidy and genome composition.**

Key words: Banana, plantain, genomes, ploidy.

INTRODUCTION

Modern bananas and plantains originated from inter- and intra-specific hybridization of two wild diploid ($2n = 2x = 22$) species, *M. acuminata* and *M. balbisiana* that possess the A and B genomes, respectively (Simmonds, 1962). Edible bananas have either 22, 33 or 44 chromosomes representing diploid, triploid and tetraploid cultivars (Stover and Simmonds, 1987). These cultivars have a wide range of genome permutations including AA, (Simmonds, 1962). Karamura et al. (1998) reported that the diploids AA gave rise to AAA triploids by meiotic BB, AB, AAA, AAB, and ABB. Other combinations such as ABBB, AAAB and AABB are also thought to occur in South East Asia (Richardson et al., 1965). Intra-specific hybridization between various subspecies of *M. acuminata* produced a range of extant diploid cultivars Simmonds (1962). Karamura et al. (1998) reported that the diploids AA gave rise to AAA triploids by meiotic chromosome restitution while interspecific hybridization between AA types (and perhaps AAA) and *M.balbisiana* (BB) resulted into various AAB and ABB types of today.

Classification of bananas into genome groups is based on a scoring system that takes into account the relative contribution of the A and B genome characteristics in the constitution of any given cultivar (Stover and Simmonds, 1987). Although widely and generally adopted for many years, this method is only as complete as the information available when they are constructed (Karamura, 1998).

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There are numerous cultivars and clones in Borneo, Indonesia and Vietnam that have not been classified (Robinson, 1996; Danh et al., 1998) with many differences existing within their genome groups. These difference may be due to the A genome from different wild subspecies of *M. acuminata* and or somatic mutations (Karamura, 1998).

Pillay et al. (2000) described the various RAPD DNA markers that are specific for the A and B genomes and can be used to identify the genomic composition of a wide range of *Musa* cultivars and hybrids. The advantages of these markers include (i) they do not rely on scoring morphological traits, (ii) can be used at any stage of the life cycle of the plant, and (iii) provides an objective way for genome classification in *Musa*. The International Institute of Tropical Agriculture (IITA) maintains a large germplasm collection of banana and plantains in southeast Nigeria and in Uganda.

The ploidy and genome composition of these plants were deduced primarily from scoring of their morphological characteristics and most of these plants were obtained as *in vitro* propagules (Vuylsteke and Swennen, 1992). Although the exact time these plants spent in culture is unknown, induced variation in ploidy is always a concern in such plants. The practical limitations inherent in breeding a perennial crop like *Musa* include the long life cycle and extensive land requirements makes it imperative that the breeder is acquainted with basic information such as the ploidy and genome composition of the breeding material. An accurate assessment of the ploidy and genome composition using reliable techniques is worthwhile. Currently,new and rapid reliable molecular techniques are available to verify the ploidy and genome composition of these plants.. The objectives of this study were to determine the genomic composition of the IITA *Musa* germplasm collection using RAPD markers and estimate the DNA ploidy levels by cytometric analysis.

MATERIALS AND METHODS

Plant material

The plant material for this study (Table 1) was obtained from our *Musa* germplasm collection at the International Institute of Tropical Agriculture, Onne Research Station, southeast Nigeria and from our Namulonge Research Station in Uganda. Cultural conditions are the same as those described by Swennen (1990).

Ploidy analysis

The nuclear DNA content of each plant was analyzed by flow cytometry to estimate the ploidy levels following procedures described by Pillay et al. (2000). Briefly, the cell nuclei were isolated by chopping the midrib tissue from young leaves with a sharp razor blade in cold OTTO I buffer (0.1 M citric acid monohydrate, 0.5% Tween 20). The suspension of nuclei was filtered through a 50 µm nylon mesh to remove large cellular material. The nuclei were stained in OTTO II buffer (0.4 M Na_2HPO_4, supplemented with 4 µg/ml DAPI, 4',6-diamidino-2-phenylindole). The fluorescence of the nuclei was analyzed with a Partec flow cytometer (Partec GmbH, Munster, Germany), at a rate of 50-60 nuclei per sec. To standardize the ploidy analyzer, the gain was adjusted so that the diploid peak represented by nuclei from *M. acuminata* ssp. burmanicoides, Calcutta 4, was set at channel 50. Under these conditions, the triploid peak is expected at channel 75 and a tetraploid peak at channel 100. At least 5000-10,000 nuclei were analyzed for each sample. Three leaf samples were tested from each of four plants of each accession.

Chromosome counts and genome composition

Karyological analysis to establish chromosome numbers in root-tip meristems were carried out as described by Pillay and Adeleke (2001). Chromosome counts were done for only those plants which the ploidy levels obtained in this study differed from those reported in previous studies. On the other hand, the genome composition of each plant was determined using RAPD primers that are specific for the A and B genomes in *Musa* (Pillay et al., 2000). Briefly eighty 10-mer primers were used to amplify DNA from *Musa acuminata* spp. *burmannicoides* (Calcutta 4) (AA genomes) and *M. balbisiana* (BB genomes). Two primers A17 and D10 from Operon Technologies (Alamedia, Calif) produced bands unique to 'Calcutta 4' and were considered specific to the A genome. These bands did not appear in *M. balbisiana*. Similarly, primer A18 produced three bands that were specific to the B genome and absent in 'Calcutta 4. In the latter case bands identifying whether a plant had either one or two B genomes were observed. Together with ploidy analysis the genome composition of 40 genotypes were identified with these markers. PCR-RFLP of the ribosomal DNA internal transcribed spacers also provides markers for the A and B genomes in *Musa* (Nwakanma et al., 2003). These markers were tested with 17 of the genotypes used in this study and the genome compositions were same as those identified by the RAPD markers.

RESULTS AND DISCUSSION

This study aimed at providing an accurate assessment of the ploidy and genome composition of the IITA *Musa* germplasm collection that comprises of over 300 genotypes from various regions of the world. Prior to this study, both the ploidy and genome composition of the germplasm were assessed on the basis of morphological characteristics. This study showed that at least 8% of plants differed in their ploidy status and/or genome composition from those reported in earlier literature both formal and informal (Table 1). 'Pisang awak', 'Foulah 4' and 'Nzizi' were previously classified as triploids (Stover and Simmonds, 1987, ITC document). Using flow cytometry, this study showed that they are tetraploids (Figure 1). Chromosome counts from root tip cells showed 2n = 44 for 'P. awak', 'Foulah 4' and 'Nzizi' (Figure 2a). Similarly, cultivars that were previously classified as diploids including 'Too' (Figure 2b) 'Toowoolee' (Figure 2c) and 'Sukali ndizi' were found to be triploids in our analysis. Although these plants constitute a small percentage of the banana germplasm, it is clear that morphology alone could not be used to determine ploidy or genome composition of bananas. An alternative explanation for the differences in ploidy levels of some of these genotypes is they may be represented

Table 1. *Musa* species and accessions used for ploidy analysis and genome composition (* shows new ploidy levels).

No	Germplasm	Ploidy*	A17 band	A18a band	A18b band	Genome composition
1	Aivip	2x	+	-	-	AA
2	Borneo	2x	+	-	-	AA
3	Colatino Ouro	2x	+	-	-	AA
4	Diploid basilian	2x (3x)*	+	-	-	AAA
5	Djum metek	2x	+	-	-	AA
6	Djum tau	2x	+	-	-	AA
7	Figue Sucree	2x	+	-	-	AA
8	Galeo	2x	+	-	-	AA
9	Gulum	2x	+	-	-	AA
10	Guyod	2x	+	-	-	AA
11	Gwanhour	2x	+	-	-	AA
12	Heva	2x	+	-	-	AA
13	Long Tavoy	2x	+	-	-	AA
14	*M. acuminata* Calcutta 4	2x	+	-	-	AA
15	*M. acuminata holotype*	2x	+	-	-	AA
16	*M. acuminata* hybrid (302)	2x	+	-	-	AA
17	*M. acuminata* hybrid (BS 382)	2x	+	-	-	AA
18	M. acuminata Madang	2x	+	-	-	AA
19	*M. acuminata* ssp. *zebrine*	2x	+	-	-	AA
20	*M. acuminata* ssp. *Malacccensis* Pahang	2x	+	-	-	AA
21	*M. acuminata* ssp. *Malaccensis*	2x	+	-	-	AA
22	*M. acuminata* ssp. Selangor	2x	+	-	-	AA
23	*M. acuminata* ssp. Truncata (BS 393)	2x	+	-	-	AA
24	*M. acuminata* ssp. Zebrina (Maioa)	2x	+	-	-	AA
25	*M. acuminata* ssp.truncata (BS 252)	2x	+	-	-	AA
26	*M.acuminata ssp truncata* (BS393)	2x	+	-	-	AA
27	M. acuminata ssp. Zebrina G.F.	2x	+	-	-	AA
28	M. balbisiana	2x	-	+	+	BB
29	M. basjoo	2x	ab	P	-	AA
30	M. laterita	2x	+	-	-	AA
31	*M. peekeli*	2x	-	-	-	TT
32	*M. schizocarpa*	2x	-	-	-	SS
33	Manag	2x	+	-	-	AA
34	Monjet	2x	+	-	-	AA
35	Morong Princesa	2x	+	-	-	AA
36	*Musa acuminata* (120)	2x	+	-	-	AA
37	No 110	2x	+	-	-	AA
38	Pa (musore)	2x	+	-	-	AA
39	Pa (Pathhalong)	2x	+	-	-	AA
40	Padri	2x	+	-	-	AA
41	Pisang Berlin	2x	+	-	-	AA
42	Pisang buntal	2x	+	-	-	AA
43	Pisang gigi buaya	2x	+	-	-	AA
44	Pisang jari buaya	2x	+	-	-	AA
45	Pisang lilin	2x	+	-	-	AA
46	Pisang madu	2x	+	-	-	AA

Table 1. contd.

47	Pisang mas	2x	+	-	-	AA
48	Pisang Mulik	2x	+	-	-	AA
49	Pisang songkla	2x	+	-	-	AA
50	Pisang tongat	2x	+	-	-	AA
51	Pitu	2x	+	-	-	AA
52	Pu-te-la-bum	2x	+	-	-	AA
53	S.F. 247	2x	+	-	-	AA
54	S.F. 248	2x	+	-	-	AA
55	S.F. 265	2x	+	-	-	AA
56	Saing Todloh	2x	+	-	-	AA
57	Tamai	2x	+	-	-	AA
58	Thong Det	2x	+	-	-	AA
59	Tjau Lagada	2x	+	-	-	AA
60	Tuu Gia	2x	+	-	-	AA
61	Ulungan	2x	+	-	-	AA
62	Undu Jamau	2x	+	-	-	AA
63	Unknown Dibit	2x	+	-	-	AA
64	Uwati	2x	+	-	-	AA
65	Waigu	2x	+	-	-	AA
66	Duningi	3x	+	-	-	AAA
67	Dwarf Cavendish	3x	+	-	-	AAA
68	Giant Cavendish	3x	+	-	-	AAA
69	Gros Michel	3x	+	-	-	AAA
70	Green Red	3x	+	-	-	AAA
71	Highgate	3x	+	-	-	AAA
72	Lacatan	3x	+	-	-	AAA
73	Lai	3x	+	-	-	AAA
74	Leite	3x	+	-	-	AAA
75	Marauw	3x	+	-	-	AAA
76	Muga	3x	+	-	-	AAA
77	Ouro Mel	3x	+	-	-	AAA
78	Pisang masak hijau	3x	+	-	-	AAA
79	Pisang nangka	3x	+	-	-	AAA
80	Poyo	3x	+	-	-	AAA
81	Red	3x	+	-	-	AAA
82	Red Dacca	3x	+	-	-	AAA
83	Too	2x (3x)*	+	-	-	AAA
84	Toowoolee	3x (3x)*	+	-	-	AAA
85	Wh-O-Gu	3x	+	-	-	AAA
86	Yangambi Km5	3x	+	-	-	AAA
87	IC 2	4x	+	-	-	AAAA
88	Ngern	4x	+	+	-	AAAB
89	Oura de Mata	4x	+	+	-	AAAB
90	Platina	4x	+	+	-	AAAB
91	3 hand planty	3x	+	+	-	AAB
92	75.19S	3x	+	+	-	AAB
93	85.03	3x	+	+	-	AAB
94	Abomienu	3x	+	-	-	AAB

Table 1. contd.

95	Agbagba	3x	+	+	-	AAB
96	Amou	3x	+	+	-	AAB
97	Apantu	3x	+	+	-	AAB
98	Apem Onniaba	3x	+	+	-	AAB
99	Apempa	3x	+	+	-	AAB
100	Asamiensa	3x	+	+	-	AAB
101	Atali Kiogo	3x	+	+	-	AAB
102	Banana Serpent	3x	+	+	-	AAB
103	Batard	3x	+	+	-	AAB
104	Big Ebanga	3x	+	+	-	AAB
105	Bise Egome 2	3x	+	+	-	AAB
106	Bo-Ahiu-Abue	3x	+	+	-	AAB
107	Bobby Tannap	3x	+	+	-	AAB
108	Bungaoisan	3x	+	+	-	AAB
109	Cantabalon	3x	+	+	-	AAB
110	Corne Plantain	3x	+	+	-	AAB
111	Currare	3x	+	+	-	AAB
112	Currare Enano	3x	+	+	-	AAB
113	Diby 2 off-type	3x	+	+	-	AAB
114	Didiede	3x	+	+	-	AAB
115	Dom. H. Rojo (630)	3x	+	+	-	AAB
116	Dominico 500	3x	+	+	-	AAB
117	Dominico Macho (635)	3x	+	+	-	AAB
118	Dominico rojo (641)	3x	+	+	-	AAB
119	Dwarf False Horn (62)	3x	+	+	-	AAB
120	Dwarf French	3x	+	+	-	AAB
121	Eberedia Ukom	3x	+	+	-	AAB
122	Egjoga	3x	+	+	-	AAB
123	Elarioon	3x	+	+	-	AAB
124	Essang	3x	+	+	-	AAB
125	Figue Famille	3x	+	+	-	AAB
126	French Reversion	3x	+	+	-	AAB
127	French Rouge	3x	+	+	-	AAB
128	Gabon 2	3x	+	+	-	AAB
129	Gabon 4	3x	+	+	-	AAB
130	Giant Cavendish	3x	+	-	-	AAB
131	Harton Maqueno (628)	3x	+	+	-	AAB
132	Harton Tigre (642)	3x	+	+	-	AAB
133	Horse Plantain	3x	+	+	-	AAB
134	Ihitism	3x	+	+	-	AAB
135	Kelong Mekintu	3x	+	+	-	AAB
136	Kinkala	3x	+	+	-	AAB
137	Kiogo	3x	+	+	-	AAB
138	Klue roi wee	3x	+	+	-	AAB
139	Laknau (AV-66)	3x	+	+	-	AAB
140	Lifong Liko	3x	+	+	-	AAB
141	Lysoka	3x	+	+	-	AAB
142	M. Ebanga	3x	+	+	-	AAB

Table 1.contd.

143	M009	3x	+	+	-	AAB
144	Madre-Del-Plantanar	3x	+	+	-	AAB
145	Mbeta 1 (Mysore)	3x	+	+	-	AAB
146	Mbi Egome 3	3x	+	+	-	AAB
147	Mbi-Egome 1	3x	+	+	-	AAB
148	Mbirinyong	3x	+	+	-	AAB
149	Mbirinyong G.M.	3x	+	+	-	AAB
150	Mimi Abue	3x	+	+	-	AAB
151	Moungali	3x	+	+	-	AAB
152	Moutouka 1	3x	+	+	-	AAB
153	Moutouka 2	3x	+	+	-	AAB
154	Msisa	3x	+	+	-	AAB
155	Mulolou	3x	+	+	-	AAB
156	Muracho	3x	+	+	-	AAB
157	Mzuzu-Nothing but green	3x	+	+	-	AAB
158	Nadzia	3x	+	+	-	AAB
159	Nazika	3x	+	+	-	AAB
160	Ngok Egome	3x	+	+	-	AAB
161	Nia bang	3x	+	+	-	AAB
162	Niangfeloa	3x	+	+	-	AAB
163	Nothing but red	3x	+	+	-	AAB
164	Nselouka	3x	+	+	-	AAB
165	Ntanga 3	3x	+	+	-	AAB
166	O. Ntanga 2	3x	+	+	-	AAB
167	O. Ntanga G.M.	3x	+	+	-	AAB
168	Obibit Ukom	3x	+	+	-	AAB
169	Obino L'Ewai	3x	+	+	-	AAB
170	Okoyo Ukom	3x	+	+	-	AAB
171	Orishele	3x	+	+	-	AAB
172	Osoaboaso	3x	+	+	-	AAB
173	Ovang	3x	+	+	-	AAB
174	Pisang ceylan	3x	+	+	-	AAB
175	Pisang kelat	3x	+	+	-	AAB
176	Pisang lang	3x	+	+	-	AAB
177	Platano H. 645	3x	+	+	-	AAB
178	Pome	3x	+	+	-	AAB
179	Popoulou	3x	+	+	-	AAB
180	Poyo	3x	+	+	-	AAB
181	Purple plantain	3x	+	+	-	AAB
182	Rajapuri India	3x	+	+	-	AAB
183	Red Plantain	3x	+	+	-	AAB
184	Red Plantain Hembra	3x	+	+	-	AAB
185	Rouge Deloum	3x	+	+	-	AAB
186	Silk	3x	+	+	-	AAB
187	Sukali ndizi	2x (3x)*	+	+		AAB
188	Topala	2x (3x)*	+	+	-	AAB
189	Trumay	3x	+	+	-	AAB
190	Tsambunu	3x	+	+	-	AAB

Table 1. contd.

191	Ubok Iba	3x	+	+	-	AAB
192	Ukom	3x	+	+	-	AAB
193	Valery	3x	+	-	-	AAA
194	Walungu 8	3x	+	+	-	AAB
195	Wine Plantain	3x	+	+	-	AAB
196	Zue Ekon	3x	+	+	-	AAB
197	Foulah 4	3x (4x)	+	+	+	AABB
198	Nzizi	3x (4x)	+	+	+	AABB
199	Pisang awak	3x (4x)	+	+	+	AABB
200	Ney Poovan	2x	+	+	-	AB
201	Blue Torres strait 1	3x	+	+	+	ABB
202	Bluggoe	3x	+	+	+	ABB
203	Cachaco	3x	+	+	+	ABB
204	Cardaba	3x	+	+	+	ABB
205	Dole 767	3x	+	+	+	ABB
206	Fougamou	3x	+	+	+	ABB
207	Green Red	3x	+	+	+	ABB
208	Ice Cream	3x	+	+	+	ABB
209	Lep Chang Kut	3x	+	+	+	ABB
210	Maduranga	3x	+	+	+	ABB
211	Pisang raja	3x	+	+	+	ABB
212	Pelipita	3x	+	+	+	ABB
213	Robusta 133	3x	+	+	+	ABB
214	Sabra	3x	+	+	+	ABB
215	Simili Radjah	3x	+	+	+	ABB

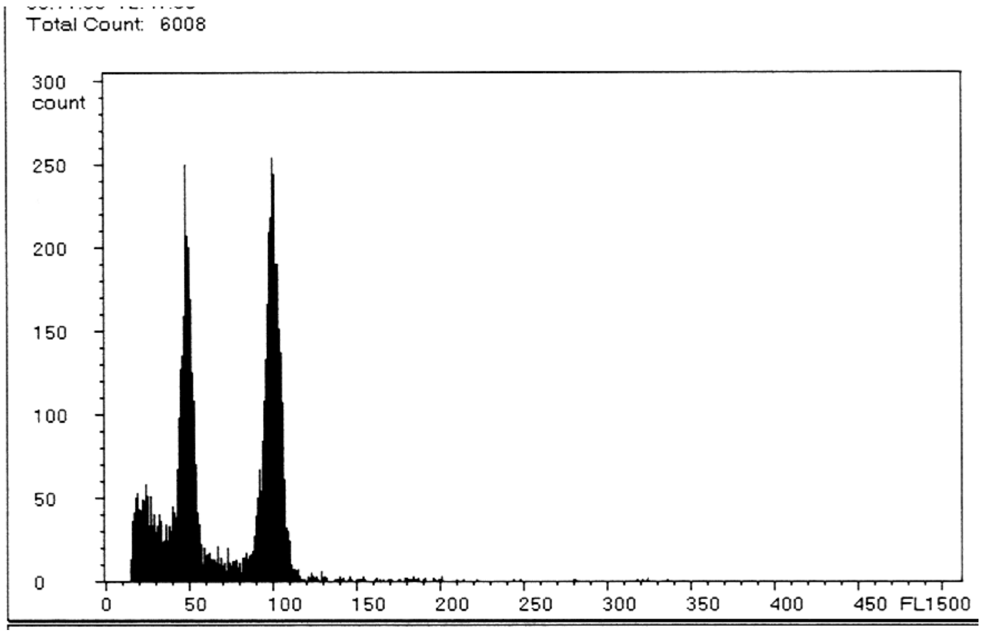

Figure 1. Representative histograms from ploidy analysis of *Musa* accessions. The figure shows peaks at channels 50 and 100 representing a diploid and tetraploid ploidy level.

Figure 2. Mitotic metaphase plates showing 44 chromosomes in "Nzizi" (a) and 33 chromosomes in 'Too' (b) and 'Toowoolee' (c).

by different cytotypes. This can only be confirmed by looking at a large number of accessions from the whole geographical range of these genotypes.

It has been previously demonstrated that total reliance on morphology alone could produce ambiguous results with regards to ploidy in *Musa* (Jenny et al., 1997; Horry et al., 1998). For example, Klue Teparot (ABBB) that was known as a natural tetraploid was found to be a triploid with flow cytometry and conventional chromosome counting (Jenny et al., 1997; Horry et al., 1998). Its genome composition was determined to be ABB (Pillay et al., 2000). Similarly, Horry et al. (1998) showed that the accessions 'Balonkawe' and 'Pisang Jambe' previously classified as tetraploids from their morphology were shown to be triploids while 'Kluai Ngoen' described as a triploid (Chomchalow and Silayoi, 1984) was reclassified as a tetraploid.

Banana improvement programs throughout the world operate on similar basic principles, although the end products of breeding may differ. This involves (i) diploid x diploid crosses and phenotypic selection to identify elite diploid parents, and (ii) triploid x diploid crosses to produce primary tetraploids initially, and then crossing these tetraploids with diploids to produce secondary triploids (see Figure 1; Pillay et al., 2001). While this scheme appears to be fairly straightforward and simple, there are a number of confounding factors. For example, many banana genotypes are known to produce 2n gametes (Ortiz, 1997). Consequently a diploid x diploid cross may not necessarily produce all diploid progeny. We have observed triploid progeny resulting from diploid x diploid crosses in *Musa* (unpublished data). Triploid by diploid crosses can produce diploids, triploids and tetraploids and in rare case pentaploids. Osuji et al. (1997) have reported aneuploids in 3x X 2x crosses in *Musa*. Banana breeding programs should consider these anomalies and routinely examine the ploidy of all plants in segregating populations to ascertain the correct ploidy of selections.

Ten major banana genomic groups are recognized in use today (Stover and Simmonds, 1987; Robinson, 1996). These include (i) AA, (ii) AAA, (iii) AAAA, (iv) AB, (v) AAB, (vi) ABB, (vii) BBB and (viii) ABBB, (ix) AAAB and (x) AABB. Bananas are classified into distinct types on the basis of their genome groups. Generally, dessert bananas and east African highland bananas are AAA, plantains are AAB while cooking bananas are ABB. Interploidy crosses between heterogenomic parents can produce an array of genotypes. For example, crosses between a plantain (AAB) and a wild diploid AA or BB parent produces progeny varying in ploidy and genome composition. These genotypes cannot be easily distinguished from morphology. Correct information on the genome composition and ploidy of parental material is essential so that the *Musa* breeder could make appropriate selections

The IITA germplasm collection was established primarily from *in vitro* propagules (Vuylsteke and Swennen, 1992). While *in vitro* material is advantageous for germplasm exchange especially in the case of banana where conventional propagules are bulky, it also has disadvantages (Daniells, 1997). One of the main problems emanating from tissue culture laboratories are variety mix-ups and miss-labeling (Daniells, 1997). Different banana varieties look similar at the test tube stage. Ploidy analysis and genome composition data are useful in identifying such mix-ups. For example the east African highland banana cultivar 'Rugondo' is classified as a triploid. Ploidy analysis of 'Rugondo' from our germplasm collection produced a peak at channel 50 suggesting that it was diploid. However, leaf samples collected from the same cultivar from Uganda showed that it was a triploid with an AAA genome composition. This example underscores the value of ploidy analysis and genome composition in *Musa*. *In vitro* culture is also widely used for rapid multiplication of plants in *Musa* breeding programs. Therefore the disadvantages of somaclonal variation, especially on ploidy, must be considered. While ploidy analyzers are not sensitive enough to detect aneuploidy in banana, more drastic ploidy changes are easily discernible. Ploidy analysis should become an integral part of any banana breeding program.

In conclusion, our study showed that flow cytometry in combination with our genome marker system is an invaluable tool in *Musa* breeding.

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