

Ploidy versus gender effects on inheritance of quantitative traits in *Musa* species

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Abstract

Several factors including ploidy and genome composition influence the inheritance of agronomic traits in *Musa* populations. Research results have indicated that gender determines the inheritance of quantitative traits in this species, but whether inheritance patterns are ploidy-dependent or gender-dependent is not known. The present research is therefore aimed at evaluating the agronomic performance of progenies of 4x – 2x versus 2x – 4x crosses in *Musa*, in order to understand how gender or ploidy affect the inheritance of quantitative traits in inter-ploidy crosses. The study was carried out at the High Rainfall Station of the International Institute for Tropical Agriculture (IITA), Onne in Southeastern Nigeria. Data on phenotypic performances of progenies of 4x – 2x and 2x – 4x crosses were collected for two seasons and analysed. The 4x - 2x crosses produced predominantly 3x progenies, whereas 2x – 4x crosses gave mostly 2x progenies. The diploid progenies of 2x – 4x crosses were shorter plants, took shorter time to flower and produced smaller bunches. The breeding values for the two groups were higher in the ratoon, than plant crop cycle. Complex microsporogenesis was observed in the parents of higher ploidy level (e.g. 4x) and may have resulted in production of predominantly monoploid gametes, $n = 1x = 1C$. Crosses between 4x and 2x parents, therefore, produced predominantly 3x progenies in 4x – 2x direction, but 2x progenies in 2x – 4x direction. Gender and ploidy determined the inheritance of characters in *Musa*, but with a preponderance of ploidy. The decision on the ploidy of individuals to serve as female or male parents in crosses is therefore critical.

Key words: Disease resistance; double reduction; genome; microsporogenesis; ploidy

Introduction

Plantains and bananas (*Musa* spp) are cultivated in sub-Saharan Africa where they provide more than 25% of the carbohydrate intake for over 70 million people. They are also important sources of revenue for smallholder farmers. The cultivated varieties are triploid that develop fruits by vegetative parthenocarpy. The fruits are highly nutritious, containing large amounts of carbohydrates and minerals such as phosphorus, calcium, and potassium as well as vitamins A and C (Sharrock & Frisson, 1998). They are easy to grow and production is relatively stable if the farms are well managed. The majority of the cultivated varieties

are susceptible to black Sigatoka disease caused by the fungus *Mycosphaerella fijiensis* Morelet (Wilson & Buddenhagen, 1986). This disease induces leaf decay and reduces the photosynthetic area leading to a reduction in yield or complete crop failure (Mobambo et al., 1993). Chemical treatment is expensive and may adversely affect the environment. Thus, the introduction of host plant resistance remains the most practical option for sustainable control of the disease especially for the resource-poor farmers. The genetic improvement of the species is therefore aimed at generating triploid (3x) hybrids that would be disease resistant while still maintaining the desirable agronomic characteristics of the landraces. Several breeding

approaches have been used in producing triploid hybrids. One such method involves tetraploidization of diploid accessions using colchicine prior to crossing with another diploid, as advocated by Tezenas du Montcel et al. (1995). An alternative method includes the generation of 3x hybrid using 2n gamete in 2x – 2x crosses via unilateral sexual polyploidization (Oselebe et al., 2001). However, tetraploid x diploid (4x – 2x) crosses remain the predominant triploid breeding scheme, although the multi-ploidy and heterogenomic structure of breeding populations results in unpredictable variation in genome size and structure across and within generations and causes complex inheritance patterns, that complicate phenotypic selection for most yield and growth traits (Ortiz and Vuylsteke, 1996). It is also expected that reciprocal crosses involving 2x female and 4x male would generate 3x individual as in other species. In breeding 3x hybrids, several factors may influence the inheritance of important agronomic traits. For example, there is a clear relationship between ploidy and morphological characters of plants in *Musa* spp (Tezenas du Montcel et al. 1995). The diploids in comparison with the triploids are small banana plants, with slender pseudostem, erect leaves, good ratooning and small bunches. According to Simmonds (1948a, b), there is a steady increase in the minimum thickness of leaves with increase in ploidy. The polyploids have more drooping leaves than the diploids, an effect which increases with increase in ploidy. Again, ploidy affects root diameter in *Musa* species. Diploid roots measured between 5.1 - 5.7 mm, triploids 6.2 - 8.5 mm and tetraploid roots about 7.4 mm (Monet & Charpentier, 1965).

Ploidy also influence phenology and yield parameters in *Musa* including plant height, bunch weight and related characters. Triploids differ from the diploids in the volume of the flower parts and of the fruits. They are more vigorous and show a high degree of gametic sterility, which was of importance to human selection (Purseglove, 1988). On the other hand, it was postulated that gender determined the inheritance of quantitative traits. Previous research (Tenkouano et al., 1998a) identified traits that were primarily inherited from male (2x) or female (4x) parents. For example, pest resistance, increased bunch weight and reduced time interval between flowering and harvest were more predictably inherited from a diploid male background. In contrast, plant height, number of leaves, and suckering behaviour would be improved by first selecting for the traits in a tetraploid female background before crossbreeding with diploid males. Whether such inheritance pattern is ploidy-dependent or gender-dependent is not known. This research was therefore aimed at a) determining the ploidy of progenies of 4x – 2x and 2x – 4x crosses and b) evaluating the agronomic performance of

progenies of 4x – 2x versus 2x – 4x crosses in *Musa* in order to establish whether gender or ploidy determine the inheritance of quantitative traits.

Materials and methods

Study Site and genetic material

The study was carried out at the High Rainfall Station of the International Institute for Tropical Agriculture (IITA), Onne, in Southeastern Nigeria (4° 43' N, 7° 01' E 10 masl). Detailed characteristics of the station have been described elsewhere (Ortiz et al., 1997). *Musa* populations used in this study were developed by IITA at the study site. This involved crosses between female-fertile triploid plantain landraces (*Musa* spp., AAB group) and male-fertile diploid accessions (AA) to obtain aneuploid and euploid progenies. Eight tetraploid (4x) and eight diploid (2x) progenies selected from these crosses (Vuylsteke et al., 1993b; Vuylsteke & Ortiz, 1995) were intermated in 4x – 2x and 2x – 4x crosses to produce the secondary hybrids used in this study. At maturity, zygotic embryos were extracted from viable seeds 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 prior to transfer to the field. Parental clones were micropropagated clonally using meristematic tissue from shoot tips.

Field Experiments

The plants were field established in a randomized complete block design (RCBD) with 2 replications. Cultural practices were similar to those used by Swennen (1990) under alley cropping with multispecies hedgerows. Agronomic data were collected for the number of days from planting to flowering, plant height at flowering (cm) measured from soil level to the point where the two highest petioles meet each other, number of standing leaves at flowering (NSLF) i.e. the total number of healthy leaves on the pseudostem at flowering; and number of youngest leaves showing necrotic spots due to black Sigatoka disease at flowering (YSLF). This was measured according to the methods of Vakili (1968) and Meredith and Lawrence (1969). The last two data were used to estimate black Sigatoka resistance termed index of non spotted leaves (INSL = [(YSLF – 1/NSLF)* 100]. INSL indicated the proportion of standing leaves that were free from infection. Other agronomic traits measured include bunch weight (kg), i.e. weight of a mature inflorescence of the plant carrying the fingers

Table 1. Analysis of variance for some phenology and yield characteristics of progenies derived from 2x – 4x and 4x – 2x crosses.

Source	DF	Time to Flowering	Plant height (cm)	Index of non-spotted leaves (%)	Bunch weight (kg)	Fruit length (cm)	Fruit circumference (cm)	Yield (t ha ⁻¹ yr ⁻¹)
Replication (R)	1	49760.6***	8933.5*	31.1	18.9*	11.1	6.3	74.0*
CrossID (CD)	18	144634.1***	16975.0***	198.3	72.2***	135.4***	55.0***	206.3***
2x - 4x versus 4x - 2x	1	455925.6***	56714.4***	373.6	323.6***	716.5***	211.7***	638.4***
Cycle ©	1	28805.8*	156198.7***	1.6	243.0***	141.0***	35.2***	594.4**
CD*C	18	4563.7	1979.7	34.3	17.7*	10.6	2.7	82.5***
Error		6724.3	1848.8	83.1	5.0	8.1	3.4	13.5
R Square		0.4	0.4	0.1	0.3	0.3	0.3	0.3
CV (%)		29.3	16.7	16.5	93.5	33.7	29.9	95.8

* **, and *** indicate significant F-test at P < 0.05, P < 0.01 and P < 0.001, respectively.

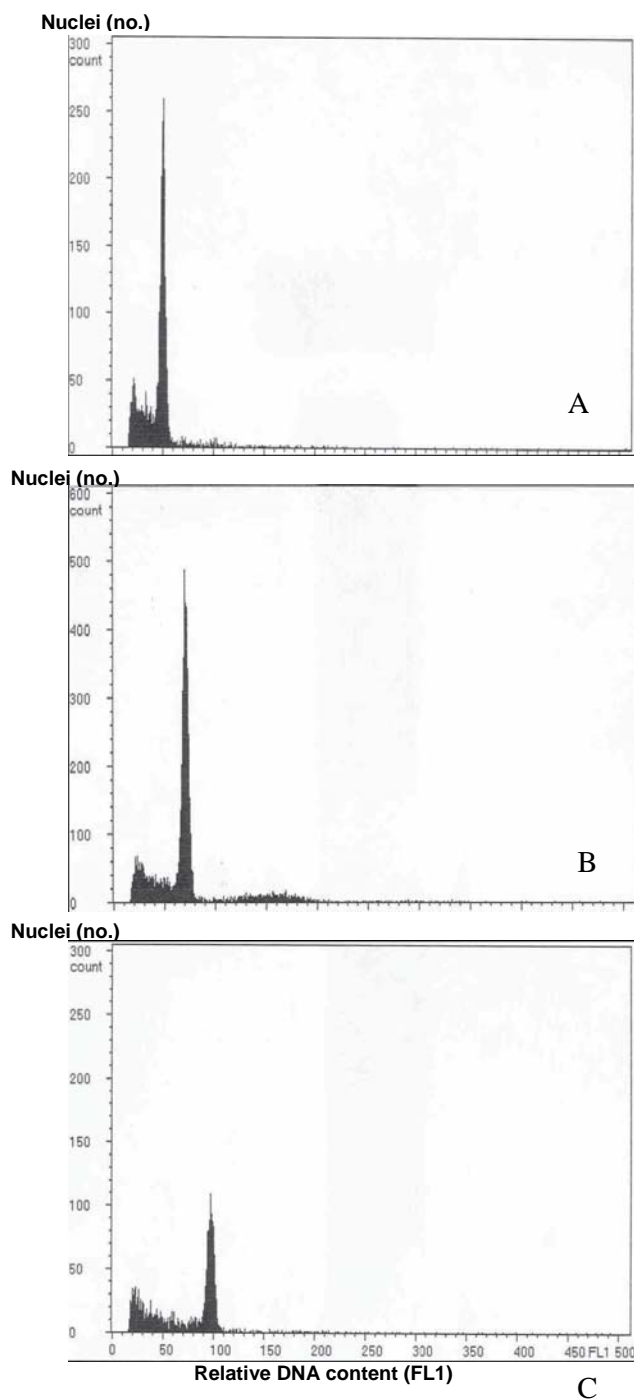


Fig 1. Typical histograms of 4-6-diamidino-2-phenylindole (DAPI)-labeled cell nuclei at pre-DNA synthesis phase (G1) from (A) a diploid, (B) a triploid, and (C), a tetraploid *Musa* sp. plant subjected to flow cytometry. FL1 indicates the fluorescence signal intensity, which stoichiometrically relates to DNA content. Oselebe et al. (2006 b).

(grouped in hands), average fruit length (cm) and fruit circumference (cm).

Data collected were subjected to statistical analysis using the general linear model (GLM) procedure in Statistical Analysis system (SAS), SAS Institute (1999), version 8. The performance of progenies from 4x – 2x and 2x – 4x crosses were assayed by means of orthogonal contrasts. Thus, the contrast was 2x – 4x hybrids versus 4x – 2x hybrids. Significant parameters were subjected to mean separation using the least significant difference (LSD) procedure at P = 0.05.

Ploidy analysis

Ploidy analysis of the field-grown plants were done as described in Oselebe et al (2006 a and b). Leaf samples were collected from the cigar (emerging tightly rolled leaf), or youngest fully expanded leaf 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 1 buffer (0.5 M citric acid monohydrate, 0.5% Tween 20) to release cell nuclei. Another 0.5 ml Otto 1 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 11 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 PAS 11 flow cytometer (Partec GmbH, Germany) whereby relative fluorescence intensities were translated into histograms corresponding to the relative DNA content, hence ploidy status, of field-grown plants (Dolezel, 1997). Two reference accessions of 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 G1 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 (Fig 1), corresponded to diploid, triploid and tetraploid plants, respectively (Pillay et al., 2000). Peak records were used to construct the frequency distribution of ploidy classes for 4x-2x and 2x- 4x crosses.

Table 2. Comparative field performance of progenies of 4x – 2x and 2x – 4x crosses grown during 1999-2001 at Onne. Data are means, minimum (Min.), maximum (Max)

Plant characteristics	2x - 4x (465)			4x - 2x (353)			LSD (P = 0.05)
	Mean	Min.	Max.	Mean	Min.	Max.	
Number of days to flowering	237.8	144.0	549.1	335.1	167.0	677.0	65.8
Plant height (cm)	246.1	130.0	395.0	271.9	115.0	430.0	39.4
Bunch weight (kg)	1.6	0.1	8.4	3.5	0.1	20.8	1.9
Fruit length (cm)	7.3	0.8	15.5	10.3	2.4	22.0	2.3
Fruit circumference (cm)	5.4	0.8	9.6	7.4±0.1	1.0	12.2	1.5
Yield (t ha ⁻¹ yr ¹)	2.8	0.2	15.4	5.2	0.1	38.9	3.1

Results

The 4x-2x crosses produced predominantly 3x progenies (94.1 %). About 3.0 % of the hybrids were 2x, 2.4 % were 4x, while 0.3 % were 5x (pentaploid). Progenies recovered from 2x–4x were mainly 2x (96.2 %), while 3.8 % were 3x hybrids (Fig 2). Significant differences ($P < 0.001$) between progenies of 4x – 2x and 2x – 4x crosses were recorded for number of days to flowering, plant height (cm), bunch weight (kg), fruit length (cm) and fruit circumference (cm). There was no significant difference between the two groups for index of black Sigatoka resistance (Table 1). Crop cycle effect was significant for all the traits studied except for index of black Sigatoka resistance. The within crosses variance was significant for most traits except index of black Sigatoka resistance, while interaction between crop cycle and the different crosses were significant for bunch weight only (Table 1). The progenies of the 2x – 4x crosses, which were mainly diploids, took shorter time to flower (238 days) with a minimum of 144 days (approximately, five months). On the other hand, triploid progenies of 4x – 2x crosses took longer time to flower (335 days) with a minimum of 167 days, approximately six months (Table 2). Progenies of the 2x – 4x crosses were shorter plants (246.1 ± 2.0) compared to progenies of 4x – 2x crosses (271.9 ± 3.1). Comparatively, however, they had higher mean values for index of black sigatoka resistance (56.9 ± 0.5) that was not significantly different from the value obtained for progenies of 4x – 2x crosses (53.4 ± 0.4). Diploid progenies of 2x - 4x crosses had lower yield and smaller bunches (2.8 ± 0.1 kg and 1.6 ± 0.1 kg, respectively) compared to progenies of 4x - 2x crosses (5.2 ± 0.3 kg, 3.5 kg) which were mainly triploid individuals. There was significant crop cycle effect on the performance of plants in this experiment. Progenies of 2x – 4x crosses flowered earlier at plant crop (218.5) than the ratoon crop cycle (262.0). The same trend was observed for most of the traits studied (Table 3).

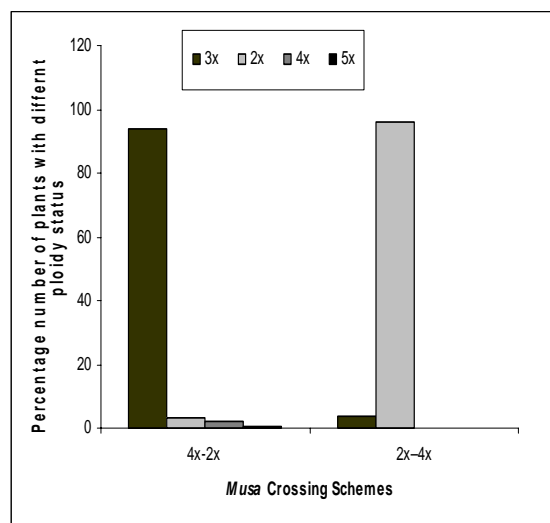


Fig 2. Percentage number of plants and ploidy status of progenies from 2x – 4x and 4x – 2x crosses in *Musa* populations.

The progenies derived from 2x – 4x and 4x – 2x crosses also had higher values for bunch weight and its components in the ratoon crop than in the plant crop.

Discussion

It was of utmost importance to establish the differences in the progenies derived from 2x – 4x and 4x – 2x breeding schemes because of the far-reaching implications for generating triploid hybrids in *Musa*. This would likely clarify whether gender or ploidy determine the inheritance of quantitative traits in this species. Theoretically, these two approaches to breeding triploid hybrids, 4x – 2x, 2x – 4x could be adopted. A third possibility that utilizes unilateral sexual polyploidization via 2n gametes in 2x – 2x crosses could also be assessed (Oselebe et al., 2001).

Table 3. Crop cycle main effect on mean performances of progenies derived from 2x – 4x versus 4x – 2x crosses

Plant characteristics	Plant crop (Cycle 1)		Ratoon crop (Cycle 2)	
	2x - 4x	4x - 2x	2x - 4x	4x - 2x
Number of days to flowering	218.5 ± 3.1	328.2 ± 6.5	262.0 ± 5.0	351.6 ± 12.6
Plant height (cm)	224.3 ± 1.9	254.7 ± 3.5	273.4 ± 2.8	313.0 ± 4.8
Bunch weight (kg)	1.3 ± 0.1	2.8 ± 0.2	2.1 ± 0.1	5.1 ± 0.4
Fruit length (cm)	6.8 ± 0.1	9.7 ± 0.2	7.8 ± 0.2	11.7 ± 0.4
Fruit circumference (cm)	5.2 ± 0.1	7.2 ± 0.1	5.7 ± 0.1	8.0 ± 0.2
Yield (t ha ⁻¹ yr ¹)	2.5±0.1	4.0±0.2	3.2±0.2	7.9±0.8

The present result and those of Oselebe et al., (2006 a) have indicated that progenies of 2x – 4x crosses were mainly diploids with diploid characteristics, while progenies of 4x – 2x crosses were mainly triploids. Diploid by tetraploid (2x – 4x) crosses can only generate diploid progenies if the 4x male parent produced predominantly 1x (monoploid) gametes. This was also highlighted by Oselebe et al., (2006 a) who indicated that complex microsporogenesis in parents of higher ploidy level (e.g. 4x) results in production of gametes with varying number of chromosome sets, predominantly $n = 1x = 1C$, as a result of double reduction or little or no pairing among the chromosomes due to the divergence of the genome sets in the genotype of the 4x parent. Crosses between 4x and 2x parents therefore produced predominantly 3x progenies ($n = 2x = 2C + n = 1x = 1C$), while 2x – 4x crosses produced 2x progenies ($n = 1x = 1C + n = 1x = 1C$). The strategic role of microsporogenesis in determining the ploidy of individuals from interploidy crosses is therefore re-emphasized.

A clear relationship exists between genome size and morphological characters of plants in *Musa* species (Tezenas du Montcel *et al.*, 1995). For example the diploids, in comparison with the triploids, were smaller plants with smaller pseudostem, and smaller bunch. The expression of most of the agronomic traits correlated with ploidy. Diploid progenies of 2x – 4x crosses took shorter time to flower, but with lower yield and bunch weight compared to the progenies of 4x – 2x crosses. The higher yield and bunch weight of the triploids, which was still higher in the ratoon crop than in the plant crop, was attributed to higher values for fruit length and fruit circumference. Vandehout et al. (1995) had reported similar results where tetraploids produced heavier bunch compared to diploids. Higher order epistatic interactions may therefore be very important in determining the actual performance of hybrids. According to Peloquin and Ortiz (1992), tri- allelic and tetra-allelic interactions are important for maximizing yield in vegetatively propagated crops.

Significant crop cycle effects in both cross types for most of the phenology, yield and its components is explained in terms of time and resources available to the plants in the ratoon, than plant crop cycle. According to Stover and Simmonds (1987), there was a longer period of vegetative growth in the ratoon, than in the plant crop. This therefore offered the plants more time for biomass production in the ratoon crop cycle (Evans, 1993). Again, resources available to the ratoon crops were more in terms of organic matter from decay of pseudostem and leaves from harvested plant crops and fertilizer (Baiyeri and Ortiz, 1997; Lahav and Turner, 1989). Thus, these environmental factors led to better expression of most of the characters in the ratoon than plant crop cycle. This may therefore denote that selection based on traits of interest in this type of population could be better in the first ratoon, than in the plant crop. Similarly, the non-significant difference for index of black Sigatoka resistance recorded in this experiment could be as a result of several cycles of selection over the years leading to an increase in disease resistance among selected hybrids.

From the foregoing, therefore gender (i.e. male or female) and ploidy determine the inheritance of characters in *Musa*, with a preponderance of ploidy effects. The decision on the ploidy of individuals to serve as female or male parents in crosses is critical. This will determine the product of microsporogenesis and in turn determine the ploidy levels of progenies from such crosses.

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