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Predicting hybrid performances from interploidy crosses in *Musa* species

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Abstract

Models for predicting hybrid performance of progenies from crosses have been developed, but genetic parameters for different *Musa* populations would have to be estimated from experimental data. Determination of these parameters would be dependent on defining the relationships of progeny to parents in different crosses and identifying parental contributions to offspring. The objective of this work was to estimate genetic relationships based on meiotic mechanisms in parents, and redefine models for predicting hybrid performance for different inter-ploidy crosses in *Musa*. The study was carried out at the High Rainfall Station of the International Institute of Tropical Agriculture in Onne (4º43' N, 7º01'E, 10 m above sea level). A breeding population of approximately 2150 individuals was developed from crosses involving tetraploid (4x) and diploid (2x) parental lines. The diameter of pollen grains from parental lines was measured under a light microscope, while the ploidy status of the breeding populations was determined by flow cytometry (FCM) analysis. Tetraploid lines had a higher mean pollen diameter than diploids. However, there were no differences between the diameter of "n" pollen of the 4x and 2x lines. 4x - 2x crosses produced predominantly 3x progenies, whereas 2x – 4x crosses gave mostly 2x progenies, suggesting a pattern of unequal contribution of the parents with respect to 3x progeny from 4x – 2x crosses, but equal parental contribution to 2x progeny from 2x – 4x crosses. The knowledge of parental contributions to progeny was used to redefine models for predicting hybrid performance from interploidy crosses in *Musa*.

Introduction

Bananas and plantains (*Musa* spp) are major staple foods in the tropic and sub-tropic regions of the world, where they provide about 25 % of the carbohydrates and 10 % of the daily calorie intake for over 70 million people. Cultivated varieties are mainly triploids (2n = 3x = 33), derived from intraspecific hybridisations within *M. acuminata* (A genome) and interspecific hybridisation between *M. acuminata* and *M. balbisiana* (B genome). Plantains and bananas have a peculiar biology, which tend to impede rapid breeding progress. These include irregular meiotic behaviour due to triploidy (Ortiz and Vuylsteke, 1996), ploidy and genome variation in crosses (Tenkouano et al., 1998a) partly due to variation in ploidy level of gametes produced during micro and megasporogenesis in male and female parents (Oselebe and Tenkouano, 2009; Oselebe et al 2006a). Others include long generation cycle (18 – 24 months from seed to seed), large land requirement ($6m^2$ per plant) and cost of extensive field evaluations for large populations. Hypothetically, the breeding of triploid hybrids of *Musa* could be approached from several angles: 4x – 2x, 2x – 4x and 2x – 2x crosses. However, in these crosses, genome and ploidy variation would be common and may complicate the ability to predict both the outcome of crosses and the performance of product of such crosses, for important economic traits (Tenkouano et al., 1998a). An understanding of the pattern of these variations is critical to the formulation of procedures for the prediction of hybrid performance prior to complex field evaluations that may be time consuming and costly. The prediction of hybrid performances is difficult due to lack of appropriate genetic models. In most cases, hybrid performances are predicted using genetic models based on disomic inheritance of traits in families derived from nested or factorial mating schemes. In this instance, progeny performance is predicted from mid-parent mean with the assumptions that there is an equal contribution of parents to their offspring, that the parents are inbred and unrelated and that the traits under consideration are strictly determined by additive inheritance (Panter and Allen, 1995; Bernado et al., 1996). These assumptions may not be suitable for *Musa* populations, because maternal and paternal contribution to the triploid offspring may not be equal (Tenkouano et al., 1998a and b). In addition, the assumption of inbred and unrelated parents may not be met in most *Musa* breeding populations, due to a 'background noise', resulting from the development of the breeding lines from very few parents as a result of low male and female fertility of the landraces. The determination of genetic parameters for predicting hybrid performance in *Musa* would therefore, be dependent on defining the relationships of progeny to parents in different crosses and an understanding of the parental contributions to offspring. This will lead to the definition of efficient strategies to obtain triploid clones from tetraploid-diploid, diploid-tetraploid or diploid-diploid parents. The objective of this work was to estimate genetic relationships based on meiotic mechanisms in parents and redefine models for predicting hybrid performance for different crosses.

Table 1. Pedigree details and utilization classes of lines used as parents

Selection No.[a]	Breeding No.[b]	Pedigree[c]	Utilization class
2x - 2x Crosses			
Females (2x)			
OSH52	TMB2x8084-2	SH3362 x 'Calcutta 4'	Banana
OSH53	TMB2x8075-7	SH3362 x 'Calcutta 4'	Banana
OSH60	TMB2x9717-7	'Manang' x 'Calcutta 4'	Banana
OSH63	TMB2x9839-2	'Calcutta 4' x Padri	Banana
Males (2x)			
OSH10	TMP2x1297-3	'French reversion' x 'Calcutta 4'	Plantain
OSH29	TMP2x2829-62	'Bobby Tannap' x 'Calcutta 4'	Plantain
OSH31	TMP2x4400-8	'Bobby Tannap' x 'Calcutta 4'	Plantain
OSH38	TMB2x5105-1	'Pisang lilin' x 'Calcutta 4'	Banana
OSH53	TMB2x8075-7	SH3362 x 'Calcutta 4'	Banana
4x - 2x Crosses			
Females (4x)			
OSH20	TMPx1658-4	'Obino l'Ewai' x 'Pisang Lilin'	Plantain
OSH27	TMPx2796-5	'Bobby Tannap' x 'Pisang Lilin'	Plantain
OSH35	TMPx4698-1	'Obino l'Ewai' x 'Calcutta 4'	Plantain
OSH45	TMPx6930-1	'Obino l'Ewai' x 'Calcutta 4'	Plantain
OSH46	TMPx7002-1	'Obino l'Ewai' x 'Calcutta 4'	Plantain
Males (2x)			
OSH38	TMB2x5105-1	'Pisang lilin' x 'Calcutta 4'	Banana
OSH49	TMB2x7197-2	SH3362 x 'Long Tavoy'	Banana
OSH52	TMB2x8084-2	SH3362 x 'Calcutta 4'	Banana
OSH60	TMB2x9719-7	'Manang' x 'Calcutta 4'	Banana
OSH63	TMB2x9839-2	'Calcutta 4' x 'Padri'	Banana
2x - 4x crosses			
Females (2x)			
OSH12	TMP2x1448-1	'Obino l'Ewai' x 'Calcutta 4'	Plantain
OSH29	TMP2x2829-62	'Bobby Tannap' x 'Calcutta 4'	Plantain
OSH62	TMP2x9839-1	'Calcutta 4' x Padri	Plantain
Males (4x)			
OSH32	TMPx4479-1	'Bobby Tannap' x 'Calcutta 4'	Plantain
OSH42	TMPx5706-1	'Obino l'Ewai' x 'Calcutta 4'	Plantain
OSH51	TMPx7579-1	'Obino l'Ewai' x 'Calcutta 4'	Plantain

[a]The OSH designation is for Onne Selected Hybrids, indicating hybrids selected at Onne breeding station of the International Institute of Tropical Agriculture.

[b]Serial cross number with prefix TMP or TMB stands for "Tropical *Musa* Plantain' (plantain-derived hybrid) or 'Tropical *Musa* Banana' (banana-derived hybrid).

[c]The accessions 'Bobby Tannap' and 'French reversion' are triploid AAB plantains that are susceptible to black Sigatoka: 'Calcutta', 'Manang', 'Padri' and 'Pisang Lilin' are diploid *Musa acuminata* (AA) accessions from south-east Asia that are resistant; SH3362 is a bred diploid hybrid from the Fundación Hondureña de Investigaçion Agricóla (FHIA) in Honduras.

Materials and methods

Study site and genetic materials

The study was carried out at International Institute for Tropical Agriculture (IITA), High Rainfall Station Onne, Southeastern Nigeria (4^o 43' N, 7^o 01' E 10 masl). IITA Onne Station is found in the densely populated Niger Delta region of Rivers State. Detailed characteristics of the station have earlier been described (Ortiz et al., 1997). The progenies for evaluation in this experiment were developed from two mating schemes: factorial-mating involving 4x - 2x and 2x - 2x parents and bi-parental crosses involving 2x and 4x individuals as female and male parents, respectively. *Musa* primary tetraploid plantain hybrids including OSH20, OSH27, OSH35, OSH45 and OSH46 were crossed to five primary diploid banana hybrids OSH38, OSH49, OSH52, OSH60 and OSH63 in a 4x-2x breeding scheme. In addition, four diploid banana hybrids: OSH52, OSH53, OSH60, and OSH63 were crossed to three diploid plantain hybrids: OSH10, OSH29, OSH31 and two diploid banana hybrids: OSH38 and OSH53. Also three diploid plantain hybrids: OSH12, OSH29 and OSH62 were crossed to three tetraploid plantain hybrids: OSH32, OSH42, OSH51, in a 2x – 4x crossbreeding scheme. These parental lines were developed at the study site. Pedigree details of these *Musa* lines are shown in Table 1. At maturity zygotic embryos were extracted from seeds and germinated in vitro. The parental clones were micropropagated clonally using meristematic tissues from shoot tips. Seedlings were acclimatized in the nursery prior to transfer to the field. Progenies and parental clones were established in the field under alley cropping with parental clones as controls.

Pollen Analysis

Pollen samples were collected from parental clones between 7:30 and 10:30 a.m. Pollen grains were dislodged from the

Table 2. Sources of variation, degrees of freedom and mean square estimates for pollen characteristics in diploid and tetraploid parental accessions

Source of variation	DF	Pollen Quantity	Stainability %	Pollen diameter (µm)	
				Mean	Mode
Sample (S)	2	0.3	2.0	281.3	228.2
2x vs 4x (P)	1	0.5	489.3*	2827.4**	615.1
S * P	2	0.5	19.6	26.3	556.3
Error	105	0.5	30.7	248.9	353.8
R-Square		0.1	0.1	0.1	0.1
CV (%)		32	5.5	12.7	15.4

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

stamen, spread on a microscope slide and stained with acetocarmine glycerol gelly (Marks, 1954). One hundred pollen grains each (from four microscopic fields) from eight slide preparations per parent were observed under a Leitz Diaplan binocular light microscope (×400 magnifications). The diameters of ten randomly selected deeply stained grains were measured with the aid of a graduated eyepiece. The most frequent pollen diameter was considered to be that of normal haploid (n) pollen size. A mathematical approach to the evaluation of pollen diameter was used (Veronessi et al., 1988; Tondini et al., 1993). The diameter of n pollen of tetraploids was comparable to 2n pollen diameter of diploids and bigger than n pollen of diploids. Pollen grains were classified as unreduced (2n) or giant pollen, if they had about 1.26 (i.e. those ≥ 160 µm) or 1.59 times the linear dimensions of haploid pollen, respectively (Darlington, 1937). The number of haploid (n), diploid (2n) and giant (4n) pollen grains was recorded per parent. This was used as an estimate of the products of microsporogenesis and an indication of the range of gametes produced by parents of specific ploidy levels. Data on pollen quantity, variability and size were collected and subjected to the analysis of variance using the GLM procedure in SAS.

Ploidy analysis

The ploidy status of the breeding populations was determined by flow cytometry (FCM) analysis following the procedures outlined in Oselebe et al (2006b), based on the postulate that variation in DNA content can be equated with variation in ploidy status. To release cell nuclei, about 50 mg of *Musa* leaf mid-rib was chopped with 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). A further 0.5 ml Otto 1 buffer was added and the suspension mixed with a pipette. The suspension of the released nuclei was filtered through a 50 µm nylon mesh and kept at room temperature. To stain the DNA, 2ml Otto 11 buffer (0.4 M anhydrous Na_2HPO_4) containing 4µg/ml DAPI (4-6-diamidino-2-phenylindole) was added. Fluorescence of DAPI-stained nuclei was analysed using a Partec PAS 11 Flow cytometer (Partec GmbH, Germany) to ascertain the nuclear DNA content and determine the ploidy status of the genotypes (Dolezel 1997). The Flow cytometer was standardized using a known diploid 'Calcutta 4'and a known triploid ('Obino l'Ewai') as an internal reference standard (Pillay et al., 2000). Gain of the instrument was adjusted 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 and setting kept constant during analysis of samples prepared from the breeding populations to compare their peak or histogram (Fig.1). Peaks on higher channels would indicate higher ploidy levels (Pillay et al., 2000). Ploidy levels of the progenies of crosses

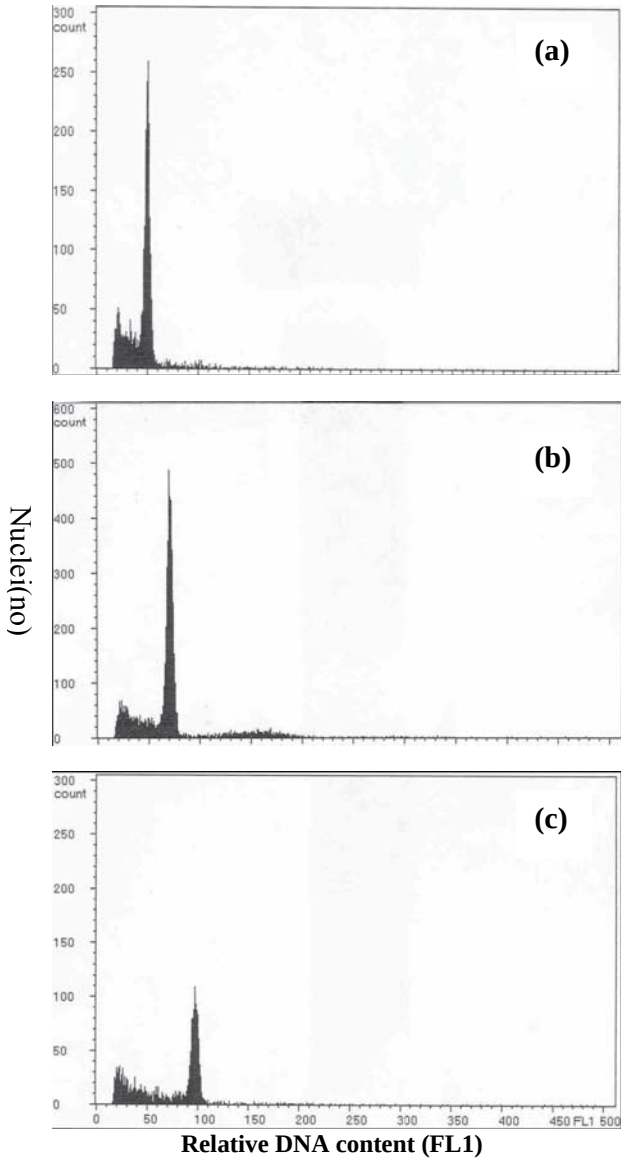

Fig 1. Culled from Oselebe et al., (2006 b). Typical histograms of 4-6-diamidino-2-phenylindole (DAPI)-labeled cell nuclei at pre-DNA synthesis phase (G1) from (**a**) a diploid, (**b**) a triploid, and (**c**), a tetraploid *Musa* sp. plant subjected to flow cytometry. FL1 indicates the fluorescence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

Table 3. Pollen characteristics of diploid (2x) versus tetraploid (4x) hybrids used as parents

Ploidy level	Pollen Quantity (rating)[A]	Stainability %	Pollen diameter (µm)	
			Mean	Mode
2x	2.3	97.7	117.7	118.4
4x	2.1	93.7	128.2	123.7
LSD (P = 0.05)	ns	2.2	6.2	ns

A: 1 = poor, 2 = medium and 3 = abundant. ns = not significant

were recorded and ploidy frequencies expressed in percentages.

Predicting Hybrid Performance

A modified mid-parent approach for predicting hybrid performance was proposed (Tenkouano et al., 1999b), based on the following equation:

$$Hij = \frac{ci\,(\,1 + fii\,)\,Pi + cj\,(1 + fjj\,)\,Pj}{ci\,(1 + fii) + cj\,(1 + fjj)} \times \left(1 - Ln\ \frac{2 - fij}{\sqrt{(fii)\,(fjj)}}\right)$$

Where, H_{ij} indicates the expected value of the hybrid produced from the ith and jth parents. P_i and P_j are the observed values of the ith and jth parent, respectively. There are two components of the equation: a) an additive component, which is the weighted average of parental phenotypes, and b) a multiplicative component, which reflects heterotic or inbreeding effects. The terms c_i and c_j are the relative contributions of parents i and j, respectively to their progeny. In a disomic situation, genomic contribution of parents to the offspring are equal, i.e. $c_i = c_j$ (Panter and Allen, 1995; Bernado et al., 1996). In *Musa* however, it is postulated that secondary triploid *Musa* hybrids receive two chromosomes from their tetraploid maternal parent for every chromosome donated by their paternal diploid parent. Therefore, ($c_i \neq c_j$) provided that 2n gametes were not formed. The terms f_{ii} and f_{jj} represents the probability of two alleles being identical at any locus in parents i and j, respectively and is indicative of their homozygosity (inbreeding) level. F_{ij} refers to the coefficient of relationship (similarity) among parents i and j. The three terms: f_{ii}, f_{jj} and f_{ij} may be calculated using pedigree information, or molecular data, or a combination thereof (Tenkouano et al., 1998). The logarithm term included is analogous to Nei's (1972) formula for the calculation of genetic distance and reflects the postulate that the difference between the expected progeny performance and the mid-parent value would vary as a function of genetic distance among parents. If parents were similar genetically, the logarithm term would approach zero and the performance of the progeny would be expected to approach the mid-parent value for the trait under consideration. This is the case from the progeny obtained from selfing an inbred line, which will be expected to express the same genotypic value as the inbred line. In contrast, crossing two unrelated parents should lead to an F_1 that expresses hybrid vigour, the magnitude of which would depend on parental contribution and inbreeding status. In other to predict hybrid performances, estimates of relationships between parents and progenies generated from pollen analysis of parental lines and ploidy analysis of progenies from different crosses would be substituted in the modified mid-parent formula for predicting hybrid performances in inter-ploidy crosses.

Results and discussion

The aim of breeding schemes involving 4x and 2x lines in *Musa* populations is to produce 3x progenies that have superior agronomic and post-harvest characteristics. In order to predict the outcomes of 2x – 2x, 2x – 4x and 4x – 2x breeding, it was important to examine the frequency distribution of ploidy classes in gametes produced by the 4x and 2x parents which would be an indication of their contribution to their offspring. This was based on the postulate that parental contribution to progeny in 4x – 2x crosses would be 1:1 for 2x offspring, 2:1 or 1:2 for 3x offspring and 3:1 or 2:2 for 4x offspring. Significant differences among 2x and 4x genotypes were observed for mean pollen diameter ($P < 0.01$) and pollen stainability ($P < 0.05$), but not for pollen quantity and most common pollen diameter (Table 2). There were no significant sample effects over time and sample by genotype interaction was also not significant. The tetraploid parents had a higher mean pollen diameter (128.2 µm) than the diploid lines (117.7 µm), while the diploids had a higher stainability value (97.7%), compared with the tetraploids (Table 3). The significant difference in pollen diameter between diploid and tetraploid parents is analogous to their DNA content (Ortiz et al., 1998) and supports the postulate of Dessaw (1988) that pollen derived from normal microsporogenesis should contain chromosome numbers that reflect the ploidy state of the sporophyte. Based on this postulate, the mean pollen diameter should therefore be a function of the genome size, because the nucleus and cytoplasm increase as chromosome number increases. The higher stainability value of the diploids in this study may be indicative of normal microsporogenesis, balanced gametes and hence pollen viability. Only well rounded and deeply stained pollen grains were counted and regarded as being viable in line with Ortiz et al., (1998). On the other hand, meiotic irregularities in 4x microsporogenesis may have led to the production of higher number of unbalanced gametes, hence, lower stainability values. These irregularities may be related to pairing abnormalities during meiosis leading to unbalanced and/or aborted pollen grains, and reduced stainability. Although the 4x parents had a reduced stainability value, there was no significant difference in pollen production between them and the 2x parents. Of a particular note, however, is the non-significant difference in the most common pollen size between the two parental lines. Normally, the most common pollen size is regarded as the haploid (n) pollen of a particular clone. 2n pollen of diploids is comparable to n pollen of tetraploids and bigger than n pollen of diploids (Verronesi et al., 1988). This implies that the diameter of n pollen of tetraploids should be equal to 2n pollen of diploids, each having the same chromosome content (2n = 2C for 2x parent; n = 2C for 4x parent). Non-significant difference in the n pollen diameter between the diploids and the tetraploids may therefore indicate a predominance of 1x pollen in the tetraploid male parent which could lead to ploidy polymorphism in progenies from crosses in *Musa* populations having 4x as a male parent.

Table 4. Theoretical expectations for the number of chromosome sets in gametes and zygote from crosses between tetraploid and diploid *Musa* lines

Gametes		4x parents					
		1	2	3	4	6	8
2x parents	1	2	3	4	5	7	9
	2	3	4	5	6	8	10
	4	5	6	7	8	10	12

Diploid individuals may produce gametes with 1, 2, or 4 times the basic chromosome set, considering only euploid products. Whereas predominating gametes are normal (n = 1C) from diploid *Musa* lines, restitutional gametes do occur and are widespread among accessions and hybrids (Ortiz, 1997), resulting from failure of first meiotic division (first divisional restitution [FDR]) or second meiotic division (second divisional restitution [SDR]) or of both, referred to as double restitution (Tavoletti et al., 1991). From the later cases, 2n gametes (2n = 2C) and 4n gametes (2n = 4C) are formed. Ortiz (1997) also suggested that at least one dominant allele may be involved in the inheritance of 2n pollen in *Musa*. Other factors that may contribute to the formation of gametes with varying chromosome sets (n = 1x = 1C, n = 3x = 3C and n = 6x = 6C) include spindle disorientation, abnormal cytokinesis, incomplete homeology of the genomes that make up the genotype of the line or highly divergent genome sets which could reduce pairing (Ude et al., 2002). Disjunction in this later case for different configurations may lead to the formation of gametes with 1n, 3n and 6n chromosomes. Upon fusion of gametes from 4x and 2x individuals, progenies with 1 to 12 sets of the basic chromosome complements may therefore be recovered (Table 4).

Ploidy variation in progeny

Based on analysis of microsporogenesis, two postulates was drawn: crosses between 4x and 2x parents would predominantly produce 3x progenies in the 4x – 2x direction and 2x progenies in the 2x – 4x direction, assuming that macrosporogenesis was normal. Progenies from 2x – 2x crosses would also produce predominantly 2x progenies. Results of the FCM analysis from this study were complementary. 4x - 2x crosses produced predominantly 3x progenies, whereas 2x – 4x crosses gave predominantly 2x progenies. Individuals recovered from 2x – 2x crosses were predominantly diploids (Fig 2). This suggested a pattern of unequal contribution of the parents with respect to 3x progeny (n = 2x = 2C by the 4x parent plus n = 1x = 1C by the 2x parent) from 4x – 2x crosses. The reverse was the case for 2x – 4x crosses where parental contribution was equal with respect to the progeny (n = 1x = 1C plus n = 1x = 1C).

Predicting hybrid performance

Progress in breeding for higher yield in *Musa* would be dependent on identifying and accessing genes and gene combinations most likely to produce higher heterosis and the selection of appropriate parental lines whose combinations are likely to lead to hybrids of the desired ploidy level and heterosis. The modified mid-parent approach (Tenkouano et al., 1999b) could be used to estimate hybrid performance with the substitution of estimates of parental contribution based on present results. In 4x – 2x crosses which constitute the most reliable means of generating triploid hybrids (Oselebe et al 2006b), in which 3x progenies are predominantly recovered (> 90 %), and in conformity with the assumption of normal meiosis in both parents, hybrid performance could be predicted using the following equation:

$$Hij = \frac{2(1 + fii)\,Pi + (1 + fjj)\,Pj}{2(1 + fii) + (1 + fjj)} \times \left(1 - Ln\ \frac{2 - fij}{\sqrt{(fii)\,(fjj)}}\right)$$

The above equation may also apply for 4x – 4x crosses based on our findings that 4x male parents produces predominantly n = 1C gametes.

Other specific applications of this formula may suffice for different crosses. In disomic situations, there may be two options, which would be dependent on segregation outcomes during meiosis. In a breeding scheme that utilizes 2n gametes produced through unilateral sexual polyploidization in 2x – 2x crosses to produce 3x progenies, unequal parental contribution (n = 1x = 1C + n = 2x = 2C or n = 2x = 2C + n = 1x = 1C) would be expected. In this breeding scheme, hybrid performance could be predicted using the equation below:

$$Hij = \frac{(1 + fii)\,Pi + 2(1 + fjj)\,Pj}{(1 + fii) + 2(1 + fjj)} \times \left(1 - Ln\ \frac{2 - fij}{\sqrt{(fii)\,(fjj)}}\right)$$

In a normal situation, parental contributions to progeny will be equal from 2x – 2x crosses (Panter and Allen, 1995; Bernado et al., 1996), and hybrid performance could be predicted thus:

$$Hij = \frac{(1 + fii)\,Pi + (1 + fjj)\,Pj}{2} \times \left(1 - Ln\ \frac{2 - fij}{\sqrt{(fii)\,(fjj)}}\right)$$

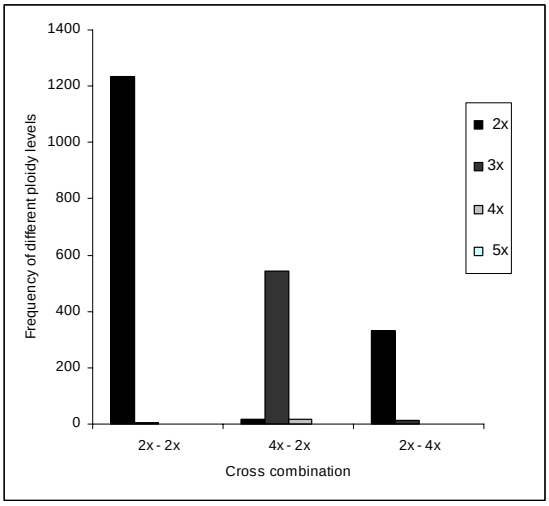

Fig 2. Ploidy composition of progenies from 2x – 2x, 2x - 4x and 4x - 2x crosses

This equation would also apply to 2x – 4x crosses where equal contribution of parents to their progeny is expected. This is substantiated by present results in relation to 4x microsporogenesis and the preferential survival of monoploid gametes. In all the cases, the terms f_{ii} and f_{jj} denotes the probability that two alleles are identical at any locus in parents i and j, respectively; f_{ij} represents the coefficient of relationship among the parents. All the three values would be

calculated from various combinations of pedigree and molecular data (Tenkouano et al., 1999a). The ability to predict hybrid performance prior to field trials would reduce the cost of *Musa* breeding, both in terms of labour, time and money. It is worth noting that, it may not be easy to predict accurately the outcome of any cross in *Musa*. However, outcomes with the greatest percentage most of the time may be taken as the normal situation.

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