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# Paternal Contribution to Banana (*Musa sapientum* L.) & Plantain (*Musa paradisiaca* L.) Progenies & Progeny Ploidy Composition in a Polycross Mating System Using RAPD & Flow Cytometry

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author AT designed the study, author MP provided support and assistance in the flow cytometry and marker analyses and author VW carried out the field and lab work, collected and analyzed data and developed the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** A 4x – 2x polycross mating design of 4 tetraploid female parents was established to determine paternal contributions of 3 diploid male parents to resulting progenies, their ploidy composition and genetic diversity of synthetic hybrids.
**Study Design:** The polycross mating design comprised 2 blocks having both maternal and paternal selections, with seed parents replicated at 12 plants per clone. Each crossing block had 31 plants of each of the three male parents.

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**Place and Duration of Study:** International Institute of Tropical Agriculture (IITA) High Rainfall Station, Onne (4º51'N, 7º03'E, 10 m above sea level), Rivers State, South-South Nigeria for a period of 24 months.
**Methodology:** At maturity of maternal parents (TMPx 2796-5; TMPx 1658-4; TMPx 5511-2; and TMPx 7152-2), fruit bunches were harvested, ripened and the seeds extracted. Hard seeds obtained were germinated *in vivo* in seed trays and emerging seedlings transplanted to perforated nursery bags. At 12 weeks, DNA was extracted from candle leaf for RAPD analysis of 80 progenies and the 3 pollen parents. Ploidy status of progenies was determined using flow cytometry method.
**Results:** There was significant unequal paternal contribution to *Musa* polycross progenies with 3 maternal parents; TMPx 2796-5, TMPx 5511-2, and TMPx 1658-4. Two of the 3 paternal parents had progenies with all 4 maternal parents while TMB2x 5105-1 did not have any progeny with TMPx 2796-5. Progenies exhibited 4 ploidy levels with frequency differing with each female parent: TMPx 7152-2 produced 100% 3x progeny; TMPx 5511-2, 63% 3x and 37% 2x; TMPx 2796-5, 91% 3x and 9% 2x and TMPx 1658-4, 82% 3x, 9% 2x, 6% 4x and 3% 5x. The 5x progeny was recorded in the first ratoon crop. The second ratoon crop had only triploids.
**Conclusion:** The high frequency of 3x progenies from all maternal types in this study, suggests the effectiveness of the polycross mating design in *Musa* improvement.

*Keywords: Musa spp.; tetraploid hybrids; heterosis; ploidy composition; RAPD.*

## 1. INTRODUCTION

In *Musa* research, an important benefit of 4x – 2x breeding is that the tetraploid (4x) hybrids are high-yielding and resistant to black Sigatoka, while diploid (2x) hybrids have low yield potential but are resistant to black Sigatoka disease. The tetraploid hybrids are both female and male fertile but this often reduces fruit quality due to the presence of seeds in the pulp. However, this characteristic of the tetraploid hybrids facilitates the production of a large number of seeds when they are crossed with other accessions. To restore seedlessness, crosses are made between the primary 4x and 2x hybrids to produce secondary 3x hybrids. This allows the development of superior plantain varieties by incorporating additional traits into the triploid plantain hybrids. Choice of crosses must therefore take into account parental diversity and intra-family variation leveraging on heterosis and allowing pyramiding of resistance genes from different diploid progenitors [1,2,3]. This 4x-2x breeding approach has been successfully used for genetic improvement of potato [4,5]. Use of the polycross mating system and attendant open pollination is a common practice in forage crop breeding. The use of this approach has been reported [6,7] to efficiently enhance inter-mating between 4x and 2x accessions and synthesize triploids in Meadow fescue (*Festuca pratensis* Huds.) and Pensacola bahia grass (*Paspalum notatum* Flügge). Scientists have recommended the polycross as a useful tool for quantitative plant breeding analysis [8,9]. More recently, researchers have tested for unequal paternal contributions using nuclear and chloroplast SSR markers in polycross families of radiata pine [10]. Others have employed the polycross and AFLP molecular marker parental selection to produce superior progenies in fescue [11]. However, the polycross mating system is relatively uncommon in *Musa* breeding. The term 'polycross' is used to designate the pollination system based on natural random inter-mating of diverse genotypes grown together in isolated blocks. It is therefore, a systematic arrangement of several improved seed and pollen parents, and this provides a mechanism for simultaneous introgression of desirable genes into breeding populations. This mating system maintains the genetic variability of the materials and accumulates superior traits from a number of selected parents and is suitable for plants in which genotype by environment interaction may be critical [12,13,14]. Scientists have advocated utilizing open pollination in polycross breeding systems that include selected tetraploid and diploid parents, according to their combining ability, for further improvement of the *Musa* genome and as a way to accelerate breeding work in *Musa* [15,16]. Most of the progeny derived from such a polycross mating system are expected to be triploids as a consequence of the lower viability of pollen from tetraploids. The ploidy composition of all such progeny from 4x-2x crosses cannot be guaranteed over several seasons given the irregular meiotic behavior of the species and to unpredictable variation in genome size and structure both across and within generations [17]. It was stated [16] that it takes about 1000 seeds, which are produced after more than 1000 hand

pollinations of 200 plants (0.12 ha), to obtain one selected tetraploid plantain-banana hybrid per year. Therefore, if open pollination results in progenies that are as good as or better than hand pollinated progenies, much of the labor, cost and time involved in breeding work in *Musa* could be saved and breeding work could become less stressful, faster and without loss in efficiency [16,18]. Although maternal and paternal identity of hand pollinated progenies is often known, in a polycross mating system that depends on open pollination, with source of pollen from more than one paternal parent, the identities of both parents cannot be correctly ascertained. Therefore, the inheritance of traits from such paternal parents by supposed progenies may not always materialize. The situation is further compounded by the fact that complex inheritances have been reported for most growth and yield characteristics of *Musa* [19,20], due to the irregular meiotic behavior of the species and to unpredictable variation in genome size and structure both across and within generations [21]. Because parental performance may not accurately predict progeny performance for these traits, further genetic improvement requires control of selection over prospective male and female parents through progeny testing to determine genetic parameters and assign accessions to compatible heterotic groups. To overcome this, a genetic model for estimation of progeny performance based on the performance of the parents, their inbreeding status and relatedness to each other and their contributions to their progeny was proposed [22,23,24]. Similarly, the analysis of combining ability in 4x-2x crosses revealed that some traits were primarily inherited from the male (2x) or female (4x) parents, a finding of practical importance for parental selection in 4x-2x cross-breeding [25,26,27,28]. For example, breeding for increased bunch weight and reduced time interval between flowering and harvest should aim at accumulating favorable alleles for these traits in a diploid male background through recurrent selection prior to cross-breeding with a tetraploid female. It is important therefore to find out the paternal contributions of parents to progenies in a *Musa* polycross mating system in order to ascertain / ensure that progenies obtained from such a mating design will inherit the desirable traits through a molecular analysis using RAPD. This is a faster process than the use of morphological analysis that requires the plants to be grown to near or full maturity. Scientists have also in their analysis of paternal tree population developed DNA molecular markers (e.g. RAPD, AFLP, SSR) as an approach for studying quantitative traits [29]. Practical scenarios for using molecular markers to identify male parentage in polycross mating systems were reported previously [30] but not widely in *Musa*. Also, the increased use of molecular markers will accelerate the process of recurrent selection of improved *Musa* germplasm thereby facilitating the development of new hybrids. Some researchers [31,32,33,34] have reported the use of RAPDs in analysis of genetic variation, genetic mapping, early determination of sex, molecular phylogenetics, genetic fidelity and marker assisted selection in other plant species, etc but not in *Musa*. Estimation of general combining ability (GCA) from polycross mating system assumes equal contribution of pollen parents. Failure of this assumption leads to biased GCA estimates. The paternal contributions in controlled polycross families were shown to be unequal for *Populus* spp. [35]. In this study, a polycross mating system (open pollination scheme) aimed at producing synthetic *Musa* hybrid seeds under natural pollination in isolated plots containing tetraploid and diploid hybrids with good combining ability was established to:

1. Determine the paternal contributions of 3 male parents to resulting progenies of four female parents in a 4x-2x *Musa* polycross mating system
2. Find out the ploidy composition of resulting progenies over 3 crop cycles and the extent of genetic diversity of the resulting synthetic hybrid population when the seed (maternal) parents are tetraploid (4x) and the pollen (paternal) parents are diploid (2x).

The results shall provide valuable information on the effectiveness of the polycross system in *Musa* breeding and on the genetic diversity of resulting progeny.

## 2. MATERIALS AND METHODS

This study was carried out at the International Institute of Tropical Agriculture (IITA) High Rainfall station, Onne (4º51'N, 7º03'E, 10 m above sea level), in Rivers State, South-South Nigeria. The rainfall pattern is monomodal, distributed over a 10month period from February through December, with an annual average of 2400 mm. Relative humidity remains high all year round with mean values of 78% in February, increasing to 89% in the months of July and September. The mean annual minimum and maximum temperatures are 25ºC and 27ºC,

respectively, while insolation lasts an average of 4 hours daily [36]. The soil at the experimental site is a highly leached Ultisol derived from coastal sediments of the Niger Delta and is classified as loamy and siliceous iso-hyperthermic Typic Paleudult [37] The surface (0-15 cm) soils are well drained and high in phosphorus 60 mg $kg^{-1}$, organic matter 1.85%, but are low in total nitrogen 0.18% and also acidic with a pH of 4.6. Other nutrients are potassium 0.28 me/100 g and magnesium 0.36-me/100 g.

## 2.1 Parental Selection, Hybridization and Seedling Evaluation

The following four tetraploid (4x) seed parents (maternal parents) - TMPx 2796-5; TMPx 1658-4; TMPx 5511-2; and TMPx 7152-2 and three diploid pollen parents (male parents) - TMP2x 2829-62; TMB2x 5105-1; and SH 3362 were selected from IITA's collection. Pedigree information on the parental hybrids is provided (Table 1). Both maternal and paternal selections were established in 2 polycross mating blocks (Fig. 1a & 1b). The seed parents were replicated at 12 plants per clone, and each crossing block had 31 plants of each of the three male parents in order to generate synthetic hybrids from the polycross blocks. The male and female parents were arranged according to a checkerboard layout in which each female plant was surrounded by systematically disposed male plants. A ratio of one female to eight male plants was achieved to allow for floral synchrony and optimize random inter-pollination. The experiment was located 200 m in the south and 270 m in the east away from any other plantain and banana fields; a distance which is more than the pollen dispersal distance or isolation distance of plantain and banana. This was done in order to minimize / exclude invasion of foreign pollen. At maturity, fruits were harvested, bunches ripened and the seeds extracted from each maternal parent were established in soil in the nursery. Other data collected included, the time to flowering/ anthesis of maternal and paternal parents, seed set of maternal parents over the three crop cycles.

## 2.2 Assessment of Paternal Contribution to the Progenies from the 4x-2x Polycross Mating System using Random Amplified Polymorphic DNA (RAPD)

**Plant materials:**

Hard botanical seeds obtained from the four tetraploid maternal parents (TMPx 2796-5; TMPx1658-4; TMPx 5511-2; and TMPx 7152-2)

**Table 1. Pedigree information and agronomic characteristics of *Musa* parental lines used in the polycross mating system at IITA, High Rainfall Station, Onne, Rivers State, Nigeria**

| Maternal Parents (4x) | Source | Pedigree | Agronomic characteristics |
|---|---|---|---|
| TMPx 2796-5 | IITA | Bobby Tannap (BT) x Pisang lilin (PL) | Stable high yield and bunch weight, regulated suckering and Black Sigatoka Resistance (BSR) |
| TMPx 5511-2 | IITA | Obino l'ewai (OL) x Calcutta 4 (C4) | High yielding, big fruits, good pulp quality and colour. BSR |
| TMPx 7152-2 | IITA | Mbi Egome 1 (ME 1) x C4 | High yielding, short cycling, BSR, and field resistance to Banana streak virus (BSV) |
| TMPx 1658-4 | IITA | OL x PL | Stable high yield and bunch weight, regulated suckering and moderately resistant to black Sigatoka disease |
| **Paternal Parents (2x)** | **Source** | **Pedigree** | **Agronomic characteristics** |
| TMP2x 2829-62 | IITA | BT x C4 | Male and female fertile, good bunch, fruit parthenocarpy and BSR |
| TMB2x 5105-1 | IITA | PL x C4 | Male fertile, fruit parthenocarpy and BSR |
| SH 3362 | FHIA Honduras | SH 3217 x SH 3142 | Good bunch size, BSR and resistant to race 4 of Fusarium wilt |

*Source: PBIP [38]. Serial cross numbers with prefix TMP stand for 'tropical Musa plantain' (plantain-derived) & TMB for 'tropical Musa banana (banana-derived hybrid). Accessions 'Bobby Tannap' and & Obino l'ewai- plantain landraces are triploid AAB plantains from west Africa that are susceptible to black Sigatoka disease; SH 3362 is a diploid banana from the Fundación Hondureña de Investigaçion Agricŏla (FHIA) in Honduras. 'Calcutta 4'- wild banana, 'Pisang lilin- dessert banana (AA) from southeast Asia that are resistant; are diploid Musa acuminate; Mbi Egome is a medium French plantain cultivar*

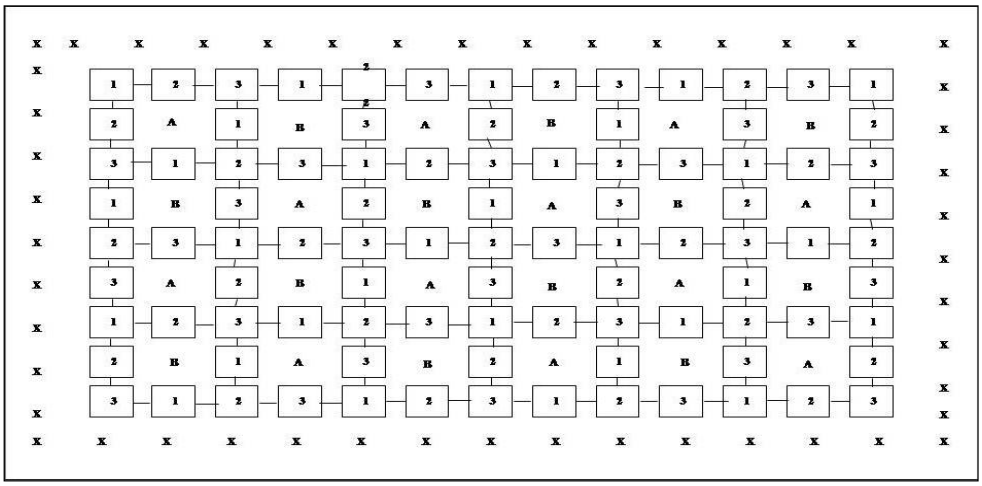


**Fig. 1a. Field layout (620 m$^2$) of *Musa* polycross scheme showing the arrangement of female 4x and male 2x parents at IITA High rainfall station Onne, Rivers State, Nigeria**

*X = Obino l'ewai (48 border plants)*

| *Tetraploid Female Parent* | *Diploid Male Parents* |
|---|---|
| *A = TMPx 2796-5* | *1 = TMP2x 2829-62* |
| *B = TMPx 5511-2* | *2 = TMB2x 5105-1* |
| | *3 = SH 3362* |

***Polycross Block 1***

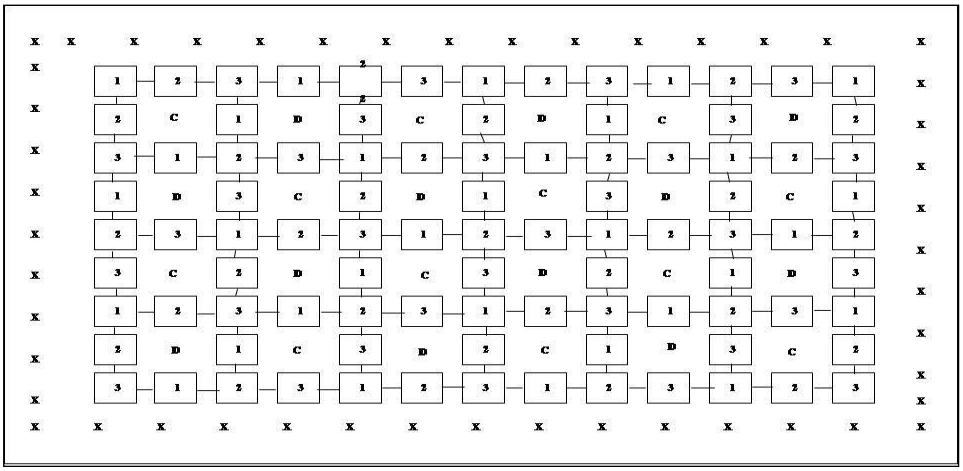


**Fig. 1b. Field layout (620 m$^2$) of *Musa* polycross scheme showing the arrangement of female 4x and male 2x parents at IITA High rainfall station Onne, Rivers State, Nigeria**

*X = Bobby Tannap (48 border plants)*

| *Tetraploid Female Parents* | *Diploid Male Parents* |
|---|---|
| *C = TMPx 1658-4* | *1 = TMP2x 2829-62* |
| *D = TMPx 7152-2* | *2 = TMB2x 5105-1* |
| | *3 = SH 3362* |

***Polycross Block 2***

used in the 4x – 2x ploycross scheme were germinated *in vivo* in seed trays. The resulting seedlings were transplanted to perforated nursery bags containing a mixture of topsoil, poultry manure in 7:1 ratio.

**Genome analysis:**

Extraction of DNA was carried out for 80 seedlings (progenies) and the 3 pollen parents used in the 4x-2x polycross scheme according to

the CTAB procedure [39,40,41]. Seven of the samples from the progenies were inadvertently contaminated, thus only samples from 73 seedlings of the 4 maternal parents from the polycross scheme were examined for paternal contribution. The DNA samples were quantified and diluted to 40 ng/µl using TE buffer. Reaction mixtures for RAPD analysis consisted of 25 µL reaction volume containing 0.2 µg DNA, 2,0 mM $MgCl_2$,0.2mM each dNTP (dATP, dCTP, dGTP, dTTP), 1.25U Taq Polymerase (U=Unit for Thermus Aquaticus*, Advanced* Biotechnologies, Surrey, U.K.) and 1.2 µM of primer in a buffer containing 75mM Tris-HCl pH 9.0 and 20 mM $(NH_4)_2SO_4$. Amplification of DNA was carried out in a Perkin-Elmer Cetus 9600 Thermal Cycler (Perkin-Elmer Life and Analytical Sciences, Inc., Wellesley, Mass.) with an initial 3min 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 and a final extension step of 7min at 72°C. About 10 µL of amplification products were separated on 1.2% agarose gels in 1x TBE buffer. Molecular weight markers included in the gel were the 100 bp ladders purchased from (Invitrogen Life Technologies Ltd., Paisley, U.K.) and pBR322 fragments (Sigma-Aldrich Company Ltd., Dorset, U.K.). The gel was stained in ethidium bromide and photographed under UV light. Two-hundred and sixty decamer primers were screened, out of which twelve (4.6%) revealed polymorphism among the male parents. Four of these primers (C-19, D-10, F-01 and J-04) gave distinct banding patterns. However, primers C19 and F1 with sequences GTTGCCAGCC and ACGGATCCTG (Operon Technologies, Alameda, Calif.) were used for genome analysis [41,42]. Amplification profiles of the progenies were compared with those of the male parents. Polymorphic bands were scored as present (1) or absent (0) for all 73 progenies. Based on the scores, a chi-square test (*P* = .05) was performed to test the hypothesis of equal contribution of paternal parents to the progenies obtained from the polycross

### 2.3 Assessment of Ploidy Composition of Progenies from the 4x-2x polycross Mating System

At 12 weeks, ploidy status of the 80 progenies was determined using the flow cytometry method (FCM). Leaf samples were obtained from the cigar leaf (emerging tightly rolled candle leaf) or youngest fully expanded leaf and immediately stored in ice-packs. In order to mechanically isolate the nuclei, 70 mg of midrib tissue of each progeny was chopped with a sharp razor blade in glass petri dish containing 1mL of ice cold Otto I buffer (0.5 M citric acid monohydrate, 0.5%Tween–20). Additional 0.5 mL of the Otto I buffer was used and the suspension was mixed and filtered through a 50 µm nylon mesh and stored at room temperature. The suspension of released cell nuclei was stained by the addition of 2 mL Otto II buffer (0.4 M anhydrous $Na_2HPO_4$) containing 4 $\mu g\ mL^{-1}$ DAPI (4-6-diamidino-2-phenylindole). Fluorescence detection was carried out with a Partec Ploidy Analyzer PA-II (Partec GmbH, Münster, Germany), and the relative fluorescence intensities were translated into histograms corresponding to the relative DNA content, reflecting the ploidy status, of the sample [43,44]. To establish the ploidy level of the progenies it was crucial to include an internal standard as a control. Three reference *Musa* species of known ploidy levels, 'Calcutta 4' (diploid banana), 'Obino l'Ewai' (triploid plantain) and a hybrid tetraploid plantain were used as internal standards and the analytical instrument calibrated so that the $G_1$ peak of nuclei isolated from the control, diploid plant was on channel 50, that of the triploid was on channel 75 while the tetraploid was on channel 100 [41,42]. The setting was kept constant during analysis of samples prepared from the 80 progenies to compare their peak or histogram to that of the reference plants. Hence, peaks appearing on various channels corresponded to diploid, triploid, tetraploid and pentaploid progenies, respectively. Peak records were used to construct the frequency distribution of ploidy status of the 80 progenies from the 4x - 2x polycross mating system. Chromosome counts were also used to reaffirm suspicion on ploidy status of any progeny.

## 3. RESULTS

### 3.1 Floral Synchrony of Paternal and Maternal Parents

The flowering patterns of the maternal and paternal parents over the 3 crop cycles are presented in Table 2. The time of flowering /anthesis in the maternal and paternal parents showed overlap /synchrony between 2 of the paternal parents (TMP2x 2829-62 and TMB2x 5105-1) and 3 of the maternal parents (TMPx 2796-5, TMPx 5511-2 and TMPx 1658-4) in the plant crop. The maternal parent TMPx 7152-2 flowered too early for the paternal parents while SH 3362 flowered too late for the maternal parents. In the first ratoon crop only TMP2x

2829-62 flowered and continued flowering within the period of flowering of 3 of the maternal parents. In the second ratoon crop TMB2x 5105-1 and SH 3362 flowered and continued flowering within the flowering period of 3 maternal parents. There were significant correlations (*P* = .05) over the 3 crop cycles between seed set of the maternal parents and days to flowering of 2 paternal parents TMP2x 2829-62 and TMB2x 5105-1 but not for SH 3362.

### 3.2 Parental Contribution to Progeny using Random Amplified Polymorphic DNA (RAPD)

Out of the 256 decamer primers tested, 12 (4.7%), revealed polymorphism among the male parents. Four of these primers (OPC-19, OPD-10, OPF-01 and OPJ-04) gave distinct banding patterns generating a total of 27 bands and the molecular markers were used to ascertain paternal (TMP2x 2829-62, TMB2x 5105-1, SH 3362) contributions to progenies of each maternal parents (Table 3).

Sixteen (59%) of these bands were monomorphic while 11 (41%) were polymorphic. Two of the 4 primers (OPC-19 and OPF- 01) confirmed bands that were specific to each male parent. Primer OPC-19 amplified three fragments with approximate molecular weights of 500-, 600- and 900-bp (base pair). The 500bp fragment was specific to TMP2x 2829-62, while the 600bp was exclusive to TMB2x 5105-1 and 900bp was definitive for SH 3362. Using primer OPF-01, 400 were specific to TMB2x 5105-1, while fragment 800 bp was unique to TMP2x 2829-62. A male parent was considered genetically related to a given progeny on the basis of observed specific male band and only progenies with unique male bands were considered (informative individuals). Out of the

**Table 2. Floral synchrony between maternal and paternal parents in the 4x – 2x polycross mating system**

| Maternal Parents (4x) | Time to Flowering (WAP*) | Duration of Anthesis (Days) | Paternal Parents (2x) | Time to Flowering (WAP*) | Duration of anthesis (Days) |
|---|---|---|---|---|---|
| **Plant Crop** | | | | | |
| TMPx 2796-5 | 51 | 4 | TMP2x 2829-62 | 48 | 32 |
| TMPx 5511-2 | 47 | 4 | TMB2x 5105-1 | 44 | 65 |
| TMPx 1658-4 | 48 | 3 | SH 3362 | 70 | 56 |
| TMPx 7152-2 | 42 | 4 | | | |
| **First Ratoon** | | | | | |
| TMPx 2796-5 | 68 | 4 | TMP2x 2829-62 | 67 | 34 |
| TMPx 5511-2 | 70 | 4 | TMB2x 5105-1 | 78 | 63 |
| TMPx 1658-4 | 65 | 4 | SH 3362 | 100 | 62 |
| TMPx 7152-2 | 69 | 5 | | | |
| **Second Ratoon** | | | | | |
| TMPx 2796-5 | 109 | 5 | TMP2x 2829-62 | 97 | 32 |
| TMPx 5511-2 | 125 | 3 | TMB2x 5105-1 | 113 | 67 |
| TMPx 1658-4 | 116 | 5 | SH 3362 | 125 | 33 |
| TMPx 7152-2 | 117 | 5 | | | |

** WAP = Weeks after Planting*

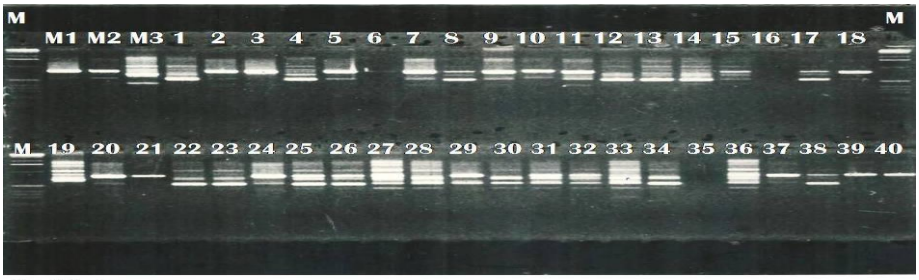
**Table 3. Random amplified polymorphic DNA amplification products (bands) for four decamer primers showing approximate size range of amplified products**

| Primers | Base composition (sequence) 5'-3' | Approximate range of fragment size (bp) | | No. of bands scored | No. of polymorphic bands (PB) | Percent polymorphism (% P) |
|---|---|---|---|---|---|---|
| | | min | max | | | |
| OPC-19 | G T T G C C A G C C | 500 | 917 | 7 | 3 | 42.9 |
| OPD-10 | G G T C T A C A C C | 572 | 858 | 6 | 2 | 33.3 |
| OPF-01 | A C G G A T C C T G | 428 | 929 | 9 | 4 | 44.4 |
| OPJ-04 | C C G A A C A C G G | 714 | 786 | 5 | 2 | 40.0 |
| Total No. of bands | | | | 27 | 11 | |

*bp = base pair*

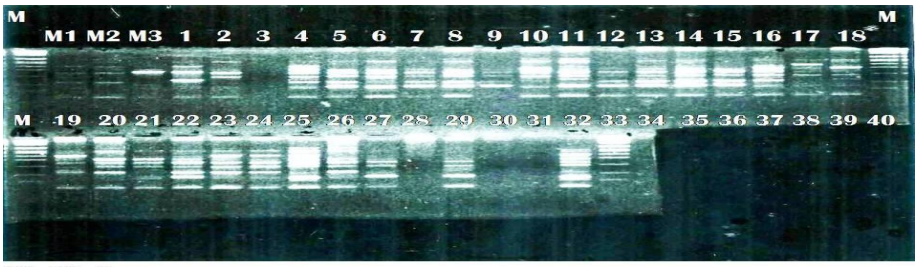
73 progenies, 11 (15%) had all their male bands missing and there were an additional 18 (25%) progenies which had bands common to two or more male parents. Consequently, these 29 progenies were excluded because they violated the concept of specific male contribution to progenies. Therefore, paternal analysis showed that only 44 (60%) progenies could be completely and definitely determined with specific paternal parents within the 73 progenies detected by RAPD markers. Arising from this, the study showed that 2 of the 3 paternal parents (TMP2x 2829-62 and SH 3362) had progenies with all 4 maternal parents (TMPx 2796-5, TMPx 5511-2, TMPx 1658-4 and TMPx 7152-2) while one paternal parent (TMB2x 5105-1) did not have any progeny with one maternal parent (TMPx 2796-5) (Table 4). The Chi square tests (p=0.5) showed that the null hypothesis, that paternal contribution to progeny was equal, should be rejected and that paternal contribution to progenies was not equal for all male and female parents. Specifically, we found significant (*P* = .05) unequal paternal contribution in the maternal parents TMPx 2796-5, TMPx 5511-2, and TMPx 1658-4.

Flow cytometry analysis (FCM) revealed four ploidy levels among the polycross progenies, the frequency of which differed with each female parent. TMPx 7152-2 produced exclusively, 100% triploids (3x) progeny. Although other females produced predominantly 3x offspring, some diploid (2x), tetraploid (4x) and pentaploid (5x) progenies were also observed (Fig. 4). In this regard, TMPx 5511-2 had the highest frequency of 2x (38.5%) along with its 3x (61.5%) progenies. TMPx 2796-5 had the lowest 2x (6.5%) with (93.5%) 3x. Only TMPx 1658-4 produced 4x (5.7%) and 5x (2.9%) along with 2x (8.6%) and 3x (82.8%) progenies. Thus, the synthetic hybrids produced from the 4x-2x polycross mating system were predominantly triploids. Over the 3 crop cycles, 2x, 3x and 4x progenies were obtained in the plant crop, the only 5x progeny was recorded in the first ratoon crop from the maternal parent TMPx 1658-4 (Fig. 5). The second ratoon crop produced only triploid progenies.




**M= Marker;**
**M1 = Male Parent 1 TMP2x 2829-62;**
**M2 = Male Parent 2 TMB2x 5105-1;**
**M3 = Male Parent 3 SH 3362;**
**Progenies = 1 - 40**

**Fig. 2. RAPD patterns of *Musa* progenies with primer OPC-19 from a 4x -2x polycross mating system at IITA High Rainfall Station, Onne, Rivers State, Nigeria**




**M= Marker;**
**M1 = Male Parent 1 TMP2x 2829-62;**
**M2 = Male Parent 2 TMB2x 5105-1;**
**M3 = Male Parent 3 SH 3362;**
**Progenies = 1 - 40**

**Fig. 3. RAPD patterns of *Musa* progenies with primer OPF-01 from a 4x -2x polycross mating system at IITA High Rainfall Station, Onne, Rivers State, Nigeria**

**Table 4. Paternal contribution to progenies of different maternal parents from 4x – 2x *Musa* polycross mating system, at IITA, High Rainfall Station, Onne, Rivers State, Nigeria**

| Maternal parents of synthetic hybrids | Total no. of progenies | Paternal contribution (%) | | |
|---|---|---|---|---|
| | | TMP2x 2829-62 | TMB2x 5105-1 | SH 3362 |
| TMPx 2796-5* | 20 | 48 | 0 | 45 |
| TMPx 5511-2* | 16 | 40 | 24 | 48 |
| TMPx 1658-4* | 32 | 34 | 25 | 33 |
| TMPx 7152-2 | 5 | 29 | 28 | 25 |

**Chi-square test, significant at P = .05*

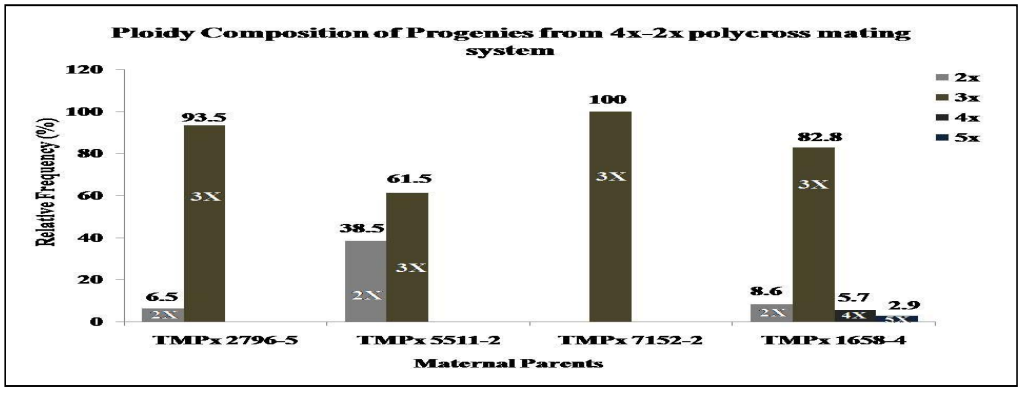



**Fig. 4. Ploidy composition of progenies from maternal parents of the 4x - 2x polycross mating system at IITA High Rainfall Station, Onne, Rivers State, Nigeria**

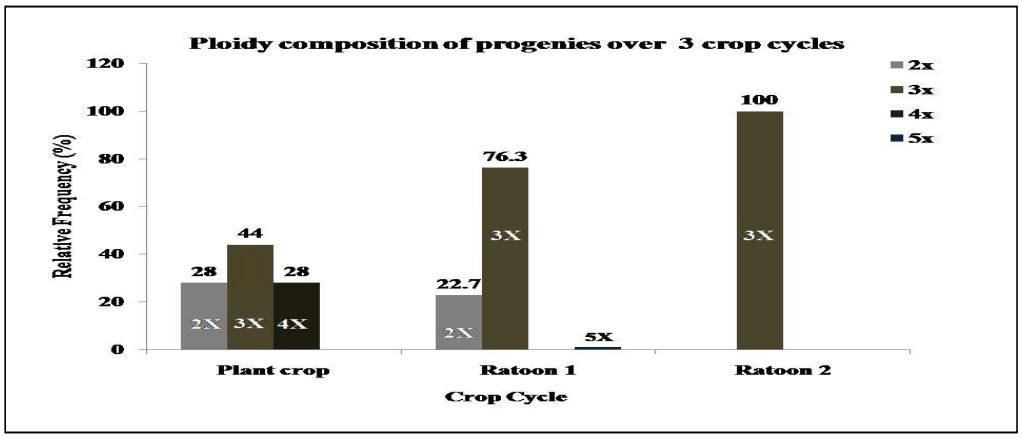



**Fig. 5. Ploidy composition of progenies from 4x - 2x polycross over 3 crop cycles at IITA High Rainfall Station, Onne, Rivers State, Nigeria**

## 4. DISCUSSION

Percentage polymorphism after use of 256 decamer primers was 41% indicating a low level of polymorphism suggesting a limited level of genetic diversity among the *Musa* spp in this study. A similar low level of polymorphism (42.0%) was detected with 400 RAPD primers in Indian accessions of *Jatropha curcus* [45] and in *Citrullus colocynthis* [46]. The primary goal of the polycross mating system is for all male parents to contribute equally to progenies of each female parent to produce synthetic hybrids. Using seven bands, the polymorphism which existed among the parents confirmed that the three male parents were genetically different. Consistent amplification of these paternal bands in some progenies using primers OPC-19 and OPF-01 indicated the paternal input in the progenies. Significant differences (*P* = .05) in the paternal

contributions to progenies were observed. Several authors who studied random mating within forest species and other crops using isozyme or molecular markers reported genetic differences, a departure from random mating [47,48,49]. The variation was attributed to differences in the fertility of the male parents. Although a small number of studies have demonstrated equal paternal contributions in some species such as *Pinus taeda* [50] and *Chamaecyparis obtuse* [51], a significant majority of studies have revealed that paternal contributions in many species in controlled polycross trials are unequal. Such species include, Sugar cane [48]; *Picea abies* [52], *Cryptomeria japonica* [53] *Populus* spp. [35] and *Silene latifolia* [54]. The causes of unequal paternal contribution have been studied in a large number of species and include genetic variations in pollen germination rate [55] or an ability to hinder other pollen by chemical interference as in Scots pine [56], high pollen production [57], pollen and pistil traits as in apricot [58]. Pollen traits can also be influenced by pistil traits that enhance pollen competition providing an ability to sort among pollen, e.g. a long style [59] a large stigmatic surface [60] or delayed stigma receptivity and fertilization [61] competition for optimal placement on the pollinator [62] and early male flowering in dioecious species as a means to compete for access to ovules of high-quality female plants [63]. Others include pollen contribution rate, pollen-pollen interactions, genetic incompatibility between male and female gametophytes, pollen tube growth rate, timing of pollen arrival on the stigma [64]. In this study, significant differences were observed in the paternal contribution to progenies of the maternal parents TMPx 2796-5, TMPx 5511-2 and TMPx 1658-4. The unequal contribution of the male parents to progenies of these female parents may be explained by the reproductive isolation in time, that is, differences in the time of flowering of the male and female parents, the duration of flowering and the synchrony of anthesis between paternal and maternal parents. Where there was overlap in anthesis between males and females, pollination and fertilization took place as in the 3 maternal parents TMPx 5511-2, TMPx 1658-4 and TMPx 2796-5 and 2 paternal parents TMB2x 5105-1 and TMP2x 2829-62. The female parent TMPx 7152-2 flowered earlier than all the female and male parents in the plant crop while the male parent SH3362 flowered much later than all the male and female parents during each crop cycle. However, due to differences in suckering behaviour of the parents, the likelihood of a male parent flowering in a given crop cycle coinciding with an early flowering female parent of the next crop cycle is very high. This was observed for the male parent SH 3362, which flowered after all other clones in the plant crop, although this coincided with flowering of the first ratoon plants of the female parent TMPx 7152-2. This points to the fact that the observed differences in paternal contribution to progeny of each seed parent, was due to temporal reproductive differences.

### 4.1 Ploidy Composition of Progenies

In this study, differences in the time of flowering of the female and male parents observed across cycles, imply that a male parent may contribute more to progenies of certain maternal parents than others. This is likely to affect the build up of a synthetic population, composed of intermating of the different female and male parents. The analysis of paternal contribution using molecular markers revealed the genetic and phenotypic diversity of the pollen parents and differences in their contribution to progenies. The polycross mating system in *Musa* revealed that progenies of the four maternal tetraploid parents are predominantly triploids. However, lower frequencies of 2x, 4x and 5x progenies were also observed. The 4x and 5x progenies were obtained from TMPx 1658-4, suggesting the occurrence of unreduced eggs (2n-gametes) in this female parent. Gametes with sporophytic chromosome number are 2n-gametes resulting from post-meiotic failures during cytokinesis.

## 5. CONCLUSION

This study demonstrates that for an effective polycross mating system in *Musa*, the selection of female and male parents that can synchronize at anthesis is important. Such parents could then be grown so that the flowering period coincides with high solar radiation and low relative humidity. The production of the highest frequency of 3x progenies from all maternal genotypes across the three crop cycles in this study suggests a major potential and effectiveness in the use of the polycross mating system in *Musa* breeding and improvement.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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