Asian Journal of Biochemistry, Genetics and Molecular Biology

2(4): 1-15, 2019; Article no.AJBGMB.52612

The Efficiency and Effectiveness of Open Pollination in *Musa* Breeding

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Authors' contributions

This work was carried out in collaboration between both authors. Author AT designed the study. Author VW carried out the field work, collected and analyzed data and developed the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2019/v2i430068
Editor(s):
(1) Dr. Ahmed Medhat Mohamed Al-Naggar, Professor, Department. of Agronomy, Faculty of Agriculture, Cairo University, Egypt.
Reviewers:
(1) Aminu Yahaya, College of Education and Priliminary Studies, Nigeria.
(2) Rudi Hari Murti, Universitas Gadjah Mada, Indonesia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/52612

Original Research Article

Received 02 September 2019
Accepted 07 November 2019
Published 14 November 2019

ABSTRACT

Aims: This field experiment was conducted to determine if hand and open pollination methods affected performances of *Musa* progenies from 4x - 2x crosses and to identify promising progenies for recurrent selection.
Study Design: The experimental design was a randomized complete block design with two replications of 6 plants per genotype.
Place and Duration of Study: 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 for 24 months.
Methodology: Two-month old seedlings of hand pollinated (6 diploid, 6 tetraploid) and open pollinated (6 diploid, 6 tetraploid) progenies, along with parental clones (2x) and (4x) of each genotype were planted at 3 m x 2 m spacing. Data on phenology, vegetative growth, yield and yield characters were collected at flowering and harvest over three crop cycles. Genotypes were partitioned into 5 clusters assayed by means of orthogonal contrasts to compare the performance of progenies from both pollination methods.

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Results: Pollination methods produced no significant (P = .05) differences, unfavourable effects or reduction in performance of economically important yield and yield components of 4x and 2x progenies of similar genotype. Some significant (P = .05) linear correlations and relationships between phenological and vegetative traits; and yield and yield components changed with pollination methods and ploidy levels but did not affect final outcomes. Promising open pollinated diploids include the early maturing TMP2x 2829-62OP; and for high yield and yield components measured, TMB2x 8084-2OP and TMP2x 1448-1OP. Promising open pollinated tetraploids include TMP4x 7002-1OP and TMP4x 2796-5OP.

Conclusion: Open pollination did not result in unfavorable effects or reduction in performance of economically important yield and yield components in progenies of similar genotypes. Therefore, open pollination could be considered for *Musa* breeding. This will reduce cost, labour, time and stress involved in *Musa* improvement.

Keywords: Musa progenies; diploid; tetraploid; hand pollination; open pollination.

1. INTRODUCTION

The overall objective of breeding programs is to select, produce and deploy the best available genetically improved materials as efficiently, extensively and effectively as possible. Conventional breeding in *Musa* is mainly conducted through sexual hybridization by hand pollination; a laborious, often stressful, costly and time consuming process in which individual female and or male flowers are covered shortly before anthesis to prevent contamination by unwanted pollen [1]. At anthesis, pollen grains from male flowers are collected and brushed against female flowers (whose flowering duration is often less than a week) usually between 0700 and 1030 hours [2]. Thus. controlled hand pollination allows intermix of genetic materials of selected elite parent plants to produce high quality, and consequently high value progenies and seeds [3,4]. This technique has been used to improve seed yields, control the level of outcrossing in seed orchards, improve breeding through knowledge of both female and male parents, achieve interspecific hybridization, and study self-incompatibility levels [5,6]. For *Musa* breeding, parental materials are often chosen on the basis of phenotypic traits [7,8] and genetic potential [9,10,11,12] as well as vegetative, phenological and reproductive growth performances [13,14]. Desirable traits such as tolerance to unfavourable abiotic conditions [15,16] and resistance to pest and diseases are also high priority [7,17,18,19]. Knowledge of the basic characteristics of dwarf and tall cultivars [20], desired ideotypes [21,22,23], results of genetic studies and combining ability tests [14] are valuable guides in the selection of male and female parents. In addition, the specific objectives of the breeding program which may combine disease/pest resistance, short stature, and interesting bunch characteristics [24,25] are critical in choice of parents. Understanding how ploidy levels and hand and open pollination methods affect the relationships between phenological traits, vegetative traits, and yield and yield components would be useful in selecting parents and guide breeding efforts for *Musa.* This is so because hand pollination is labour-intensive, costly, time consuming and stressful [26] especially for field crops with only one full growth cycle a year. According to a researcher [4] 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 labour, 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 [4,27]. Moreover, knowledge about the effects of pollination methods could provide invaluable information that can help breeders take decisions on the evaluation of parents and progenies used in breeding programs, help to identify promising offspring for cultivar selection and the development of best varieties to generate a breeding population [28,29,30]. However, some authors [31] working with eucalyptus warned that open-pollination as opposed to controlled pollination may result in a significant loss of productivity. Others [32] have suggested that loss of productivity is the result of inbreeding depression because at least 10–30% of open-pollinated seeds occur from self-fertilization. In addition to inbreeding, genetic gains from open-pollination may be reduced by high contamination from external pollen sources [33]. Contamination as high as 39% [34] and 46% [35] have been reported in open-pollination of *Eucalyptus grandis*. In their study using microsatellite markers in eucalyptus, [36] found both self and external contamination of pollen even in progenies of three types of controlled pollination methods. It is important therefore to examine if open pollination in *Musa* could also result in loss of productivity and inbreeding depression compared to hand pollination. According to Brown et al. [1] the limited progress that has been achieved in *Musa* breeding has occurred through crossbreeding approaches that involve hybridization followed by phenotypic selection among half sibs and/or full sib progenies. In order to effectively assess the true effects of hand and open pollination methods on *Musa*, this experiment set out to investigate how diploid and tetraploid progenies obtained from both pollination methods compared to each other and to their parental clones. Performance criteria chosen included phenological and vegetative traits as well as yield and yield components. This is because knowledge of vegetative traits and their correlation with economically important yield related traits is an essential first step in the development and selection of suitable genotypes for such characters that are easily measured [26]. Thus, the 4*x*–2*x* breeding scheme should aim to accumulate favourable alleles in potential tetraploid and diploid parents through recurrent selection. In his study [20] it was observed that primary tetraploid hybrids were taller, had longer production cycles but a shorter fruit filling time, and had heavier bunch weights than their diploid sibs. He further noted that there were significant differences between clones of the same ploidy within each cross. These results suggest that individual selection within ploidy will be an effective means for improving plantain germplasm. Selection for large fruit at both ploidy levels may lead to heavy fruits, thereby resulting in hybrids bearing heavy bunches. He also noticed a positive significant correlation between plant stature and bunch weight at the tetraploid level, which may indicate that selection of dwarf plantain hybrids with heavy bunches, may be a challenging endeavour. Some authors [37] have stated that yield components could serve as indirect selection criteria for yield in *Musa* hybrid populations. They also detected a higher genetic expression for most yield components during the second crop cycle (ratoon 1) in all the environments, which implies that selection should be carried out in the second crop cycle (ratoon 1). Moreover, for the average farmer in the tropics, selection of progenies that are easier to maintain are of decided advantage. Identifying the correlations between different vegetative and yield traits is essential to guide the development and selection of suitable genotypes for plantain and banana breeding. Local selections may further lead to dynamic conservation of genetic resources because farmers will preserve distinct, locally adapted, improved genotypes across environments.

This study was conducted therefore to investigate the efficiency and effectiveness of open pollination in *Musa* Breeding

2. MATERIALS AND METHODS

The 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 over a period of 24 months. 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 solar radiation/ sunshine lasts an average of 4hours daily [38]. Hand pollination was carried out as follows: the female inflorescence of the 4x and 2x hybrids were bagged at anthesis and hand pollinated with pollen from a single diploid accession (TMP2x 2829-62). For open pollination the same hybrids are grown in isolated crossing blocks with the female inflorescence exposed to allow for open pollination where natural agents effect pollination as natural pollinators. At fruit maturity (90-120 days following flower emergence), bunches from hand and open pollinated plants were harvested and ripened with ethylene for 4 days. Seeds were extracted from the ripened bunches and the embryos excised and cultured *in vitro* for 6weeks. The resulting diploid (2x) and tetraploid (4x) progeny seedlings were transferred to the nursery. Two-month old seedlings of these along with the parental clones (vegetatively propagated from parents) of each 4x and 2x genotype, were planted in the field. Thus a total of 6 diploid and 6 tetraploid parental clones, 6 diploid hand pollinated progenies, 6 diploid open pollinated progenies, 6 tetraploid hand pollinated progenies and 6 tetraploid open pollinated progenies were planted (Table 1). The experimental design was a randomized complete block design with two replications of 6 plants per genotype. Planting was done in alleys of multispecies hedgerows in an area of 2,880m^2 at a spacing of 3m x 2m [39,40]. Fertilizer was applied at the rate of 300 kg N and 450 kg K per hectare, split into 6 applications [41,42]. Weeds were controlled with Paraquat (150 ml Gramoxone in 20 L water), applied when necessary. Other cultural practices were the same as those previously described [41].

2.1 Data Collection and Statistical Analyses

Data on phenology, vegetative growth parameters, yield and yield characteristics were collected at flowering and harvest for three crop cycles (plant crop, first ratoon and second ratoon) as follows: (a) Days to flowering (DTF) - number of days elapsed from planting to emergence of the inflorescence. (b) The number of days to fruit filling (TFF) - time from the emergence of the inflorescence to harvest of the mature bunch (c) Plant height (PHT) at flowering - from the soil level to the junction of the last fully expanded leaves where the inflorescence emerged (d) Plant girth (GTH) at flowering - circumference of the pseudostem, at 100cm from the soil level (e) Height of tallest following sucker (HTFS) at flowering – gives an indication of immediacy of the next ratoon crop (f) Number of suckers (NSK) produced per plant at flowering (g) Yield and yield components. For each plant, data were recorded on bunch characteristics. The bunch was taken as the entity encompassing the node before the first hand and that of the last hand. Bunch weight at harvest (BWT) was determined by weighing using a balance (Salter Model 239). The number of hands (NH) and fingers (NF) per bunch were counted. Fruit length (FL) was taken along the convex side excluding the apex and pedicel. Fruit girth (FG) was measured as the midway circumference of the middle finger.

The yield potential (YLD, ton ha^{-1} yr^{-1}), was calculated as an estimate of bunch weight per hectare per year using the following formula:

$$YLD = \frac{(BWT \times 365 \times 1667)}{(DH \times 1000)}$$

Where: BWT = bunch weight (kg); 365 = number of days in a year; 1667 = plant population per hectare (at 3 m x 2 m spacing); DH = days from planting to harvest; 1000 = conversion of kilograms to tons. All data were subjected to the analysis of variance (ANOVA) in a randomized complete block design using the General Linear Model (GLM) of Statistical Analysis Software (SAS) version 9.1 [43] to test for significance at 5% level of significance (P =.05). To compare the performance of progenies from both pollination methods, genotypes were partitioned into 5 clusters assayed by means of orthogonal contrasts. The contrasts were: (a) parents versus (vs) progenies, (b) 2x vs 4x in parents, (c) 2x vs 4x in progenies (d) Hand pollination (HP) vs open pollination (OP) in 2x progenies, and (e) HP vs OP in 4x progenies. If a measured trait

Table 1. Parental clones and hand and open pollinated *Musa* progenies

Diploids (2x)			Tetraploids (4x)		
Parental Clones	**Hand Pollinated Progenies**	**Open Pollinated Progenies**	**Parental Clones**	**Hand Pollinated Progenies**	**Open Pollinated Progenies**
Plantains			**Plantains**		
TMP2x 1448-1CL	TMP2x 1448-1HP	TMP2x 1448-1OP	TMP4x 1658-4CL	TMP4x 1658-4HP	TMP4x 1658-4OP
TMP2x 2625-5CL	TMP2x 2625-5HP	TMP2x 2625-5OP	TMP4x 2796-5CL	TMP4x 2796-5HP	TMP4x 2796-5OP
TMP2x 2829-62CL	TMP2x 2829-62HP	TMP2x 2829-62OP	TMP4x 4698-1CL	TMP4x 4698-1HP	TMP4x 4698-1OP
Bananas			**Plantains**		
TMB2x 8084-2CL	TMB2x 8084-2HP	TMB2x 8084-2OP	TMP4x 6930-1CL	TMP4x 6930-1HP	TMP4x 6930-1OP
TMB2x 8532-1CL	TMB2x 8532-1HP	TMB2x 8532-1OP	TMP4x 7002-1CL	TMP4x 7002-1HP	TMP4x 7002-1OP
TMB2x 9839-3CL	TMB2x 9839-3HP	TMB2x 9839-3OP	TMP4x 7152-2CL	TMP4x 7152-2HP	TMP4x 7152-2OP

CL = Parental clones; HP = Hand pollination; OP = Open pollination
Serial cross numbers with prefix TMP stand for 'Tropical Musa Plantain' (plantain-derived) & TMB for 'Tropical Musa Banana (banana-derived hybrid)

was significant, the means were separated by the least significant difference (LSD) test at *P* = .05 and presented along with the 3 year means in Tables. Crop cycle main effects were examined to determine how performances differed in the plant crop, first ratoon and second ratoon crops. Linear correlation analysis (r) at 5% level of significance (*P* = .05) and 1% level of significance (*P* = .01) was performed to find out the relationships between the phenological, vegetative, yield components and yield in diploid and tetraploid progenies and to establish whether or not these relationships differed with hand and open pollination. Coefficient of determination R^2 was calculated to estimate the contribution of each of the traits to the total variation observed.

3. RESULTS AND DISCUSSION

Diploid and tetraploid progenies of *Musa* obtained by hand pollination (HP) and open pollination (OP) were compared for performance in phenological and vegetative traits, yield components and yield. The progenies were also compared to their parental clones (CL) to determine any loss in productivity. Analysis of variance (ANOVA) showed that there were significant (*P* = .05) differences in all the phenological and vegetative traits among genotypes. It also revealed varying levels of significant (*P* = .05) differences between the performances of the parents and progenies. In addition, there were some significant (*P* = .05) differences in pollination methods for some phenological and vegetative traits and in crop cycle main effects for some phenological and vegetative traits but not in the yield and yield components (Table 2).

3.1 Phenological Traits

3.1.1 Days to Flowering (DTF)

On the average, among diploid (2x) progenies, there were no significant (*P* =.05) differences between hand pollinated progenies in days to flowering and their open pollinated sibs with the exception of the open pollinated TMP2x 2829-62OP which flowered in 348days; significantly (*P* =.05) earlier by 87days than its hand pollinated sib (Table 2). The earliest flowering hand pollinated diploid was TMB2x 9839-3HP (359days). Among diploid parental clones TMB2x 9839-3CL flowered earlier than others but was only significantly (*P* =.05) different from TMP2x 1448-1CL. There was no distinct pattern of early or late flowering when diploid parental clones were compared to their hand or open pollinated progenies. Comparing the tetraploid (4x) progenies, there were no significant (*P* =.05) differences in pollination methods between hand and open pollinated sibs except between the open pollinated TMP4x 1658-4OP which flowered significantly (*P* =.05) earlier (136days) than its hand pollinated sib TMP4x 1658-4HP (Table 2). No definitive pattern of lateness or earliness in flowering was evident when tetraploid parental clones were compared to their hand or open pollinated progenies. With respect to tetraploid parental clones TMP4x 7152-2CL differed significantly (*P* =.05) only from TMP4x 1658-4CL flowering 83days later. Considering ploidy levels, generally tetraploid (4x) progenies flowered later than diploid progenies. Some progenies having similar pollination methods showed significant (*P* =.05) differences as follows: TMP2x 1448-1HP and TMP4x 1658-4HP; between TMP2x 2625-5HP and TMP4x 2796-5HP; between TMP2x 2829-62OP and TMP4x 4698-1OP; and between TMB2x 9839-3HP and TMP4x 7152-2HP; Delayed flowering in tetraploids compared to diploids have been reported [44]. At both ploidy levels, generally tetraploid parental clones flowered later than diploid parental clones. Diploid and tetraploid parental clones that flowered last were TMP2x 1448-1CL and TMP4x 7152-2CL respectively.

3.1.2 Time to Fruit Filling (TTF)

Important as days to flowering (DTF) is, some genotypes have been found to complement or compensate for late flowering with early time to fruit filling (TFF) as noted [20]. The genotype with the earliest time to fruit filling irrespective of ploidy level and pollination method among progenies and parental clones was the hand pollinated TMP2x 2829-62HP. Its TTF was significantly (*P* =.05) earlier (96days) than those of all other progenies and parental clones but did not differ significantly (*P* =.05) from those of the diploids TMP2x 2829-62OP and TMP2x 1448-1OP and tetraploids TMP4x 1658-4OP and TMP4x 7152-2HP and the parental clone TMP4x 7002-1CL (Table 2).

The tetraploid progenies with the shortest time to fruit filling were the open pollinated TMP4x 1658-4OP (109days) and the hand pollinated TMP4x 7152-2HP (111days). Among the tetraploid progenies there were no significant (*P* =.05) differences between hand and open pollinated sibs except in the case of TMP4x

1658-4HP and TMP4x 1658-4OP in which there were significant (*P* =.05) differences in TFF. As pointed out earlier however both phenological traits DTF and TFF must be consideredtogether to obtain a more holistic set of information in taking a decision for choosing promising progenies that will aid in *Musa* breeding. Therefore, taking into consideration phenological traits alone, the diploid open pollinated TMP2x 2829-62OP with 348DTF and 110TFF = 457days to maturity was best performing and most promising. Other promising diploid open pollinated progenies exhibiting fairly early maturity include TMB2x 9839-3OP and TMP2x 1448-1OP. These 3 diploid progenies could be considered for further recurrent selection in *Musa* breeding. However, if circumstances compel hand pollination, (e.g. where appropriate isolation distance cannot be applied) promising diploid hand pollinated progenies include, TMP2x 1448-1HP with 367DTF and 123TFF = 490days to maturity; TMB2x 9839-3HP with 359DTF and 133TFF = 492days to maturity and TMB2x 8532-1HP with 383DTF and 114TFF = 497 days to maturity. Among tetraploids, promising open pollinated progenies include TMP4x 1658-4OP (379.3DTF + 108.5TTF) = 488days maturity period and TMP4x 7152-2OP (421.2 DTF + 121.8 TTF) = 543days maturity period. Promising hand pollinated tetraploids include TMP4x 4698-1HP (429 DTF + 120.2 TTF) = 549.2days to maturity; and TMP4x 7002-1HP (435.3 DTF + 115.8 TTF) = 551.1days to maturity. For farmers, the earlier they can get their harvest to the consumer and charge premium prices before markets are flooded with produce that will force prices down, the better. Therefore, a differential of 2 weeks or more in time to maturity is a desirable trait to qualify a progeny for recurrent selection in *Musa* breeding especially an open pollinated progeny that can reduce cost, time, stress and labour provided yield is also appreciable.

3.2 Vegetative Traits

For plantains and bananas, plant height and girth are important because tall plants with slim girths are more prone to lodging by wind and breakage under the weight of bunches. However tall plants get more sunlight for photosynthesis but are also more difficult to harvest and require longer and more expensive stakes to keep them upright. Therefore, in selecting best performing progenies one has to consider genotypes and pollination methods that balance moderately tall plants with thick girths.

3.2.1 Plant height

Among diploid progenies there were no significant (*P* = .05) differences between open and hand pollinated progenies in height of similar genotypes, except between the open pollinated TMP2x 2625-5OP (shortest 2x progeny at 254.2cm) and the hand pollinated TMP2x 2625-5HP (tallest 2x progeny at 300.4cm) (Table 2). For diploid parental clones, there were no significant (*P* = .05) differences between them and their progenies except between the parental clone TMB2x 9839-3CL (212.5 cm) and the hand pollinated progeny TMB2x 9839-3HP (263.8 cm). For tetraploid progenies, there were no significant (*P* = .05) differences between open and hand pollinated plants of similar genotype except between TMP4x 7152-2OP (308.9 cm) and TMP4x 7152-2HP (237.5cm, shortest tetraploid progeny). Generally tetraploid parental clones were significantly (*P* = .05) taller than diploid parental clones, confirming that higher ploidy level confers greater vigour. Similar findings had been reported [20,45,46]. In this study we found tetraploid parental clones were in some instances also significantly (*P* = .05) taller than both of their open and hand pollinated tetraploid progenies (TMP4x 1658-4CL and TMP4x 7002-1CL) and in another significantly (*P* = .05) taller than only the hand pollinated progeny (TMP4x 7152-2CL). .Perhaps this could be a reflection of loss of vigour in the tetraploid progenies.

3.2.2 Plant girth

On average, 12 of the tetraploid parental clones and their tetraploid progenies had significantly (*P* = .05) thicker plant girth than diploid parental clones by 35% and their diploid progenies by 20% respectively (Table 2). Considering diploids, there were no significant (*P* = .05) differences between open and hand pollinated progenies of similar genotypes and between their parental clones (Table 2). Among tetraploid progenies, three instances are noteworthy. The hand pollinated TMP4x 1658-4HP had significantly (*P* = .05) thicker girth than the open pollinated TMP4x 1658-4OP; TMP4x 4698-1HP also had significantly (*P* = .05) thicker girth than the open pollinated TMP4x 4698-1OP. However the reverse was the case when the open pollinated TMP4x 7152-2OP had significantly (*P* = .05) thicker girth than the hand pollinated TMP4x 7152-2 HP.

3.2.3 Height of tallest following sucker

With regard to the height of the tallest following sucker which gives an indication of immediacy of the next ratoon crop, the tallest following sucker among diploids, the hand pollinated TMP2x 1448-1HP (284.2 cm) was significantly (*P* = .05) taller than two hand pollinated progenies TMP2x 2625-5HP (231.8 cm) and TMP2x 2829-62HP (238.3cm). It was also significantly (*P* = .05) taller than three open pollinated progenies: TMB2x 8084-2OP (235.4 cm), TMB2x 8532-1OP (234.9cm) and TMB2x 9839-3OP (236.8cm); and a parental clone TMB2x 9839-3CL (229.9cm) (Table 2). In addition it was significantly (*P* = .05) taller than three hand pollinated tetraploids TMP4x 1658-4HP (209.9 cm); TMP4x 4698-1HP (233.3 cm) and TMP4x 7152-2HP (209.9 cm). Overall the tallest following sucker was a tetraploid parental clone TMP4x 7152-2CL (299.5 cm) and was significantly taller than 7 other tetraploids. Generally there were no significant (*P* = .05) differences between open pollinated and hand pollinated progenies for this trait though open pollinated ones were mostly taller.

3.2.4 Number of suckers

Among diploid progenies there were significant (*P* = .05) differences in number of suckers produced between some open pollinated genotypes TMP2x 1448-1OP and TMP2x 2625-5OP; TMB2x 8084-2OP and TMP2x 2625-5OP; and between TMB2x 8084-2OP and TMB2x 8532-1OP (Table 2. Some hand pollinated progenies also differed significantly (*P* = .05) from each other: TMB2x 8084-2HP and TMP2x 2829-62HP; and between TMB2x 9839-3HP and TMP2x 2829-62HP. However there were no significant (*P* = .05) differences between hand and open pollinated progenies of similar genotype except between TMP2x 2625-5HP and TMP2x 2625-5OP only. Among tetraploid progenies there were no significant (*P* = .05) differences between hand and open pollinated progenies of similar genotype except between TMP4x 2796-5HP and TMP4x 2796-5OP. On average, tetraploid genotypes and diploid genotypes did not differ significantly (*P* = .05) in the number of suckers produced except for the hand pollinated progenies TMP4x 2796-5HP and TMP2x 2625-5HP; the parental clones TMP4x 7002-1CL and TMB2x 8532-1CL and the open pollinated progenies TMP4x 7152-2OP and TMB2x 9839-3OP (Table 2). The hand pollinated tetraploid progeny with the highest number of suckers was TMP4x 2796-5HP (4.3) while the open pollinated tetraploid progeny with the highest number of suckers was TMP4x 7152-2OP (3.7).

3.3 Yield Components and Yield

3.3.1 Bunch weight

Among diploid progenies there were no significant (*P* = .05) differences between hand and open pollinated progenies of similar genotype (Table 3). The hand and open pollinated diploid progenies with the highest bunch weights came from similar genotypes TMB2x 8084-2HP (4.6 kg) and TMB2x 8084-2OP (3.9 kg). Diploid parental clones and their progenies did not differ significantly (*P* = .05) in bunch weight. For tetraploid progenies, there were no significant (*P* = .05) differences between open and hand pollinated progenies of similar genotype except for TMP4x 7002-1OP (5.2 kg) and TMP4x 7002-1HP (2.3 kg). On average tetraploid progenies had higher bunch weight than diploid progenies. Other studies [45,46] had found tetraploids to have faster growth rates and higher yields than diploids. Three of the tetraploid parental clones, TMP4x 1658-4CL, TMP4x 7002-1CL and TMP4x 7152-2CL had significantly (*P* = .05) higher bunch weight than their hand and open pollinated progenies.

3.3.2 Number of hands per bunch

Diploid open and hand pollinated progenies of similar genotype did not differ significantly (*P* = .05) in the number of hands per bunch except for TMP2x 2829-62OP (6.2) and TMP2x 2829-62HP (4.7) (Table 3). Tetraploid open and hand pollinated progenies of similar genotype did not differ significantly (*P* = .05) in number of hands per bunch. Some diploids had significantly (*P* = .05) higher number of fingers per bunch than some tetraploids and vice versa. Generally parental clones did not differ significantly (*P* = .05) from their progenies in number of hands per bunch. The diploid progeny with the highest hands per bunch was the hand pollinated genotype TMB2x 8084-2HP (8.0) and the tetraploid progenies with the highest hands per bunch were the open pollinated TMP4x 4698-1OP (7.5) and the hand pollinated TMP4x 6930-1HP (7.5).

3.3.3 Number of fingers per bunch

Pollination method did not differ significantly (*P* = .05) in the number of fingers per bunch of tetraploid progenies of similar genotype (Table

3). In diploid progenies, pollination method did not also differ significantly (*P* = .05) in number of fingers of similar genotype except between the open pollinated TMP2x 2829-62OP (102.8) and the hand pollinated TMP2x 2829-62HP (72.3). The diploid progeny with the highest fingers per bunch was the open pollinated TMB2x 8084-2OP (140.2) and the tetraploid progeny with the highest number of fingers per bunch was TMP4x 2796-5OP (118).

3.3.4 Fruit length

Pollination method did not result in significant (*P* = .05) differences in the fruit length of diploid or tetraploid progenies of similar genotype (Table 3). On average open pollinated tetraploid progenies had longer fruits than hand pollinated ones of similar genotype whereas hand pollinated diploid progenies had longer fruits than open pollinated ones of similar genotype.

3.3.5 Fruit girth

Pollination method did not affect the fruit girth of diploid or tetraploid progenies of similar genotype significantly (*P* = .05) as shown in Table 3. On average open pollinated tetraploid progenies consistently had thicker fruits than hand pollinated ones of similar genotype; whereas hand pollinated diploid progenies had thicker fruits than open pollinated ones of similar genotype but with no distinctive pattern emerging.

3.3.6 Yield

There were no significant (*P* = .05) differences in the yield of open and hand pollinated diploid progenies of similar genotypes (Table 3). This means that pollination method did not cause significant differences (*P* = .05) in yield of diploids of similar genotype. Highest yielding diploid progenies were the hand pollinated TMB2x 8084-2HP (5.2 tons/ha) and open pollinated TMB2x 8084-2OP (4.6 tons/has). For the tetraploid progenies there were also no significant differences (*P* = .05) between open and hand pollinated progenies of similar genotype except between the open pollinated TMP4x 7002-1OP (6.9 tons/ha) and the hand pollinated TMP4x 7002-1HP (2.8 tons/ha) genotype. The highest yielding tetraploid progenies were the open pollinated TMP4x 7002-1OP (6.9 tons/ha) and TMP4x 2796-5OP (5.0 tons/ha) and the hand pollinated TMP4x 6930-1HP (5.0 tons/ha). Generally hand pollinated diploid progenies had higher yields than open pollinated diploid progenies whereas open pollinated tetraploid progenies generally had higher yields than hand pollinated progenies. On average three tetraploid parental clones yielded significantly higher (*P* = .05) than diploid parental clones. In comparing parental clones to their progenies, there was no consistent pattern of higher or lower yields. While some yielded more than their progenies, others had lower yields than their progenies. On average there were no significant differences (*P* = .05) among the diploid hand and open pollinated progenies of similar genetic background in any of the yield and yield components. On average this was partially true also of the tetraploid progenies, indicating that pollination methods clearly produced no significant differences, unfavourable effects or reduction in performance in the economically significant yield and yield components in both diploid and tetraploid progenies of similar genotype. Among the diploids, considering the yield components and yield, the promising open pollinated progenies with high bunch weight, number of hands per bunch, number of fingers per bunch, reasonable fruit length and yields are the banana genotype TMB2x 8084-2OP and plantain genotype TMP2x 1448-1OP (Table 3). Promising open pollinated tetraploid progenies are TMP4x 7002-1OP and TMP4x 2796-5OP with good bunch, high number hands per bunch, number of fingers per bunch, fruit length and reasonable yield

3.4 Main Effects of Crop Cycle

Generally, among parental clones irrespective of ploidy levels, and in progenies irrespective of pollination methods and ploidy levels, the number of days to flowering increased significantly (*P* = .05) from the plant crop to the first ratoon crop with the delay in flowering being more manifest in tetraploids (Table 4). In the second ratoon crop flowering was further delayed but more substantially in diploid parental clones and the open pollinated progenies. Time to fruit filling increased significantly (*P* = .05) from the plant crop to the first ratoon crop in both diploid and tetraploid progenies but did not differ significantly (*P* = .05) on average between both ratoon crops. Plants of parental clones and the progenies in the first ratoon crop were significantly (*P* = .05) taller than those in the plant crop. However, there were no significant differences (*P* = .05) in plant height between the first and second ratoon crops. Plants of tetraploid parents and open pollinated progenies in the second ratoon were slightly shorter than those in the first ratoon crop. Plants of parental clones and the progenies in the first ratoon crop had significantly (P = .05) thicker girths than those in the plant crop (Table 4). Plant girth between the first and second ratoon crops also increased significantly (P = .05). The plant crop, first ratoon crop and second ratoon crop revealed significant differences in some of the yield components as well as overall yield (ton/ha). The tetraploid parental clones had significantly heavier bunch weight than diploid parental clones in the plant crop and the first ratoon crop. However, in the second ratoon crop, bunch weight was not significantly different. Hand pollinated diploid progenies had significantly longer fruits than open pollinated diploid progenies only in the first ratoon crop. Also tetraploid parents had significantly longer fruits than diploid parents in the plant crop and the first ratoon crop. In the second ratoon crop, number of fingers did not differ significantly. For fruit girth tetraploid parental clones had significantly higher fruit girth than diploid parental clones in the plant crop (47%) and the first ratoon crop. Though, in the second ratoon crop fruit girth did not differ significantly. The yield (tons/ha) of tetraploid parental clones was significantly higher than the yield of diploid parental clones in the plant crop and also significantly higher in the first ratoon crop. In the second ratoon crop, yields did not differ significantly. Researchers have suggested that maximum genetic variation is exposed and is available for selection in the early generations in potato. Therefore, any increases in efficiencies of selection in these stages are likely to result in major improvements in the quality of material advancing to the later stages of selection, and to increase the likelihood of genetic improvement in cultivar production [47,48].

3.5 Correlations between Phenological and Vegetative Traits and Yield Components and Yields in Hand and Open Pollinated Diploid and Tetraploid Progenies

All 12 sets of data collected were each correlated according to ploidy level and pollination methods in all possible combinations and tested at 5% and 1% levels of significance. The correlation matrices generated contained close to 270 correlation values and we report here only the most significant ones. Significance at 5% is denoted with a single asterisk (* Significant at 5%) and significance at 1% with double asterisks (**Significant at 1%). There were notable differences and similarities in the significant linear relationships exhibited by diploid progenies under the two pollination methods. The differences were as follows: while hand pollinated diploid progenies, showed a negative and highly significant correlation between days to flowering and time to fruit filling (r = -0.966**); open pollinated diploid progenies did not show a significant correlation. Although open pollinated diploid progenies had positive and significant correlations between days to flowering and plant height (r = 0.892**); bunch weight and fingers/bunch (r = 0.838*); bunch weight and fruit length (r = 0.832*); and fruit girth and yield (r = 0.865*); hand pollinated diploid progenies did not show significant correlations in any of these. Whereas hand pollinated diploid progenies had positive and significant correlations between plant height and height of tallest sucker (r = 0.929**); plant height and fruit length (r = 0.777*); height of tallest sucker and fruit length (r = 0.899*) and height of tallest sucker and fruit girth (r = 0.874**) open pollinated diploid progenies did not show significant correlations in these traits. However hand and open pollinated diploid progenies showed significant correlations between 7 similar yield components; that is, yield and bunch weight (r = 0.968** HP; r = 0.979** OP); number of hands per bunch and bunch weight (r = 0.812* HP; r = 0.775* OP); between number of fingers per bunch and bunch weight (r = 0.915**HP; r = 0.838*OP); as well as number of fingers per bunch and number of hands per bunch (r = 0.966** HP; r = 0.941** OP); yield and number of fingers per bunch (r = 0.914**HP; r = 0.788* OP); fruit girth and fruit length (r = 0.909**HP; r = 0.906**OP); and yield and fruit length (r = 0.796* HP; r = 0.804*). In the tetraploid progenies, pollination methods resulted in marked differences and similarities as well. Hand pollinated tetraploid progenies showed significant negative correlation between days to flowering and fruit girth (r = -0.759*) but open pollinated tetraploid progenies had positive and significant correlation between days to flowering and fruit girth (r = 0.807*). While open pollinated tetraploid progenies had positive and significant correlation between days to flowering and time to fruit filling (r = 0.857*), hand pollinated ones did not show a significant correlation. Hand pollinated tetraploid progenies showed positive and significant correlations between time to fruit filling and plant girth (r = 0.826*), plant height and fruit length (r = 0.777*), height of tallest sucker and fruit girth (r = 0.842*), and between bunch weight and hands per bunch (r = 0.815*) as well as between number of hands per bunch

Table 2. Phenological and vegetative traits of parental clones (CL) and progenies from hand pollinated (HP) and open pollinated (OP) diploid (2x) and tetraploid (4x) *Musa* genotypes over 3 crop cycles at IITA High Rainfall Station, Onne, Rivers State, South-South Nigeria

Diploid (2x) Genotypes	Tetraploid (4x) Genotypes	Days to flowering		Time to fruit filling (days)		Plant height (cm)		Plant girth (cm)		Height of tallest following sucker (cm)		Number of suckers	
		2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
TMP2x 1448-1CL	TMP4x 1658-4CL	483.2	434.2	123.5	119.2	286.2	347.7	42.6	59.9	251.9	260.0	3.0	3.3
TMP2x 1448-1HP	TMP4x 1658-4HP	366.8	514.8	123.3	130.7	281.2	298.1	39.7	52.8	284.2	209.9	2.8	2.3
TMP2x 1448-1OP	TMP4x 1658-4OP	413.8	379.3	110.7	108.5	277.9	274.3	38.2	40.5	267.4	246.1	3.2	2.8
TMP2x 2625-5CL	TMP4x 2796-5CL	435.8	443.8	117.3	119.7	285.2	291.6	40.5	44.6	245.4	261.4	2.7	3.0
TMP2x 2625-5HP	TMP4x 2796-5HP	389.2	479.0	114.2	121.2	254.2	306.9	35.6	46.6	231.8	259.1	3.2	4.3
TMP2x 2625-5OP	TMP4x 2796-5OP	469.2	445.8	120.5	117.3	300.4	297.8	40.9	44.2	273.1	279.0	2.0	2.7
TMP2x 2829-62CL	TMP4x 4698-1CL	391.8	437.7	116.3	120.3	264.2	311.4	40.4	51.1	270.0	248.9	3.2	3.7
TMP2x 2829-62HP	TMP4x 4698-1HP	434.8	429.0	95.8	120.2	260.3	295.4	36.6	51.1	238.3	233.3	2.2	3.0
TMP2x 2829-62OP	TMP4x 4698-1OP	347.5	471.5	109.8	129.3	245.3	280.8	38.5	42.3	250.3	256.9	2.8	2.7
TMB2x 8084-2CL	TMP4x 6930-1CL	406.7	472.7	127.5	122.8	274.7	309.2	40.7	51.6	262.7	280.8	3.2	2.7
TMB2x 8084-2HP	TMP4x 6930-1HP	396.8	439.5	115.0	126.8	269.2	310.0	41.4	52.5	261.9	274.7	3.5	2.7
TMB2x 8084-2OP	TMP4x 6930-1OP	416.8	485.0	125.2	124.5	254.7	305.8	39.7	50.4	235.4	254.6	3.5	2.7
TMB2x 8532-1CL	TMP4x 7002-1CL	400.2	443.8	125.2	112.7	256.1	342.9	39.7	56.9	263.6	243.2	2.7	3.8
TMB2x 8532-1HP	TMP4x 7002-1HP	382.7	435.3	114.2	115.8	253.9	299.7	39.3	49.1	246.9	250.0	2.5	3.5
TMB2x 8532-1OP	TMP4x 7002-1OP	433.0	443.7	117.0	122.5	273.9	303.3	41.3	47.0	234.9	265.4	2.3	3.3
TMB2x 9839-3CL	TMP4x 7152-2CL	355.7	517.7	141.0	115.0	212.5	339.8	33.8	56.4	229.9	299.5	2.7	3.7
TMB2x 9839-3HP	TMP4x 7152-2HP	359.0	451.3	133.2	110.8	263.8	237.5	41.3	37.5	247.0	224.1	3.3	3.5
TMB2x 9839-3OP	TMP4x 7152-2OP	374.3	421.2	131.7	121.8	238.4	308.9	36.5	47.1	236.8	270.0	2.5	3.7
$LSD_{0.05}$		81.40		18.0		36.22		7.15		43.64		1.04	
R^2		0.94		0.83		0.82		0.83		0.78		0.85	
CV (%)		16.8		10.7		12.2		14.7		14.3		28.5	

Table 3. Yield components and yields of parental clones (CL) and progenies from hand pollinated (HP) and pen pollinated (OP) diploid (2x) and tetraploid (4x) *Musa* genotypes over 3 crop cycles at IITA High Rainfall Station, Onne, Rivers State, South-South Nigeria

Diploid (2x) Genotypes	Tetraploid (4x) Genotypes	Bunch weight (kg)		Number of hands per bunch		Number of fingers per bunch		Fruit length (cm)		Fruit girth (cm)		Yield (tons/ha)	
		2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
TMP2x 1448-1CL	TMP4x 1658-4CL	2.0	7.0	7.0	6.3	113.0	99.5	8.7	12.5	5.4	8.3	2.4	8.1
TMP2x 1448-1HP	TMP4x 1658-4HP	2.7	2.9	6.2	5.2	94.0	76.3	11.1	9.0	7.3	6.2	3.7	3.0
TMP2x 1448-1OP	TMP4x 1658-4OP	3.2	2.7	7.0	6.3	118.2	85.0	10.3	10.7	7.0	6.7	3.7	3.6
TMP2x 2625-5CL	TMP4x 2796-5CL	2.2	3.4	6.7	6.2	107.0	92.5	9.2	11.4	6.2	7.7	2.6	4.1
TMP2x 2625-5HP	TMP4x 2796-5HP	2.1	3.2	6.8	6.0	100.0	93.0	8.8	11.0	5.5	7.5	2.5	3.7
TMP2x 2625-5OP	TMP4x 2796-5OP	2.3	4.8	7.2	7.0	118.0	118.8	8.7	12.5	5.9	7.9	2.3	5.0
TMP2x 2829-62CL	TMP4x 4698-1CL	2.8	3.1	6.3	6.5	100.8	97.5	10.2	11.0	6.8	7.2	3.5	3.8
TMP2x 2829-62HP	TMP4x 4698-1HP	1.9	3.6	4.7	6.7	72.3	94.7	9.4	11.5	6.0	7.5	2.0	4.4
TMP2x 2829-62OP	TMP4x 4698-1OP	1.5	3.9	6.2	7.5	102.8	101.2	7.9	11.5	6.0	7.8	2.0	3.7
TMB2x 8084-2CL	TMP4x 6930-1CL	3.8	3.9	7.7	7.2	129.5	106.0	10.0	11.1	6.7	7.5	4.2	4.5
TMB2x 8084-2HP	TMP4x 6930-1HP	4.6	4.4	8.0	7.5	134.0	123.3	11.2	11.7	6.8	7.6	5.2	5.0
TMB2x 8084-2OP	TMP4x 6930-1OP	3.9	3.6	7.8	6.7	140.2	111.8	9.5	11.5	6.8	7.9	4.6	3.9
TMB2x 8532-1CL	TMP4x 7002-1CL	3.3	7.9	7.2	6.8	123.7	103.8	9.7	14.3	7.2	10.1	3.7	10.4
TMB2x 8532-1HP	TMP4x 7002-1HP	4.1	2.3	7.0	5.3	120.3	77.2	10.1	11.8	6.9	7.3	4.4	2.8
TMB2x 8532-1OP	TMP4x 7002-1OP	2.7	5.2	6.2	5.7	106.2	85.0	9.5	12.4	6.5	8.2	3.2	6.9
TMB2x 9839-3CL	TMP4x 7152-2CL	2.6	6.8	5.8	7.3	87.8	101.5	11.3	15.2	7.5	8.6	3.7	7.6
TMB2x 9839-3HP	TMP4x 7152-2HP	1.7	2.8	5.8	6.7	90.0	92.5	9.7	10.8	6.1	6.9	2.4	3.3
TMB2x 9839-3OP	TMP4x 7152-2OP	2.0	3.0	6.3	6.3	112.5	88.8	8.2	11.2	5.6	7.0	2.3	3.6
$LSD_{0.05}$		2.39		1.39		27.18		2.57		1.59		2.58	
R^2		0.81		0.72		0.72		0.80		0.82		0.96	
CV (%)		57.4		19.1		24.9		20.9		19.0		58.7	

Table 4. Main effects of Crop cycle on parental clones (CL) and progenies from hand pollinated (HP) and open pollinated (OP) diploid (2x) and tetraploid (4x) *Musa* genotypes at IITA High Rainfall Station, Onne, Rivers State, South-South Nigeria

Genotype	Days to flowering			Time to fruit filling (days))			Plant height (cm)			Plant girth (cm)		
	Plant crop	Ratoon 1	Ratoon 2	Plant crop	Ratoon 1	Ratoon 2	Plant crop	Ratoon 1	Ratoon 2	Plant crop	Ratoon 1	Ratoon 2
2x-Cl	205	397	634	117	126	132	222.2	267.7	299.5	34.5	39.2	45.2
2x-HP	204	401	560	101	122	123	234.8	264.4	292.0	35.2	38.8	42.9
2x-OP	228	388	611	112	124	121	241.8	264.4	289.4	36.5	38.5	42.5
4x-Cl	253	521	602	111	117	127	288.4	348.1	334.8	47.9	57.9	54.4
4x-HP	247	482	645	105	131	127	261.4	295.5	316.9	43.8	48.0	52.9
4x-OP	251	477	595	109	126	126	272.8	314.2	298.5	43.2	48.1	44.5
$LSD_{0.05}$	24.46			5.46			11.80			2.27		
Genotype	**Bunch weight (kg)**			**Number of fingers**			**Fruit length (cm)**			**Yield (tons/ha)**		
	Plant crop	**Ratoon 1**	**Ratoon 2**	**Plant crop**	**Ratoon 1**	**Ratoon 2**	**Plant crop**	**Ratoon 1**	**Ratoon 2**	**Plant crop**	**Ratoon 1**	**Ratoon 2**
2x-Cl	2.0	3.3	3.1	108	111	112	9.4	10.3	9.7	3.8	3.7	2.5
2x-HP	1.6	2.9	4.1	105	98	103	8.7	10.2	11.2	3.1	3.3	3.6
2x-OP	1.9	2.1	3.7	123	118	107	8.8	8.1	9.9	3.4	2.5	3.1
4x-Cl	5.8	7.6	2.6	92	93	116	14.4	14.6	8.8	8.8	7.3	2.3
4x-HP	2.8	3.9	2.9	88	83	107	11.5	11.9	9.4	4.8	3.9	2.3
4x-OP	3.1	4.6	3.8	96	80	120	11.9	12.9	10.2	5.4	4.8	3.1
$LSD_{0.05}$	0.74			8.68			0.76			0.82		

and yield (r = 0.874**); and a negative and significant correlation between number of suckers and fruit length (r = -0.850*), open pollinated ones did not show significant correlations in these traits. Conversely while open pollinated tetraploid progenies had significant positive correlations between bunch weight and fruit length (r = 0.970**), bunch weight and fruit girth (r = 0.886**); fruit length and fruit girth (r = 0.850*) and between fruit length and yield (r = 0.806*), hand pollinated ones did not show significant correlations in these traits. However, both pollination methods in tetraploid progenies had similar positive and significant correlations between plant height and plant girth (r = 0.880** HP) (r = 0.904** OP) and between bunch weight and yield (r = 0.971** HP) (r = 0.873* OP).

4. CONCLUSION

Our research has shown that pollination methods did not result in unfavorable effects or reduction in performance of economically important yield and yield components. This means that open pollination is effective and efficient enough to be considered for *Musa* breeding provided isolation distance is maintained. On average there were no significant differences in yield and yield components in open and hand pollinated diploid and tetraploid progenies of similar genotypes. Promising open pollinated diploids include the early maturing TMP2x 2829-62OP; and for high yield and yield components measured, TMB2x 8084-2OP and TMP2x 1448-1OP. Promising open pollinated tetraploids include TMP4x 7002-1OP and TMP4x 2796-5OP. These could serve as parents for recurrent selection using open pollination in order to reduce cost, labor, time and stress involved in *Musa* breeding.

ACKNOWLEDGEMENT

This work was supported by funding from the International Institute of Tropical Agriculture (IITA), Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brown A. Tumuhimbise R, Amah D, Uwimana B, Nyine M, Mduma H, Talengera D, Karamura D, Kuriba J, Swennen R. Bananas and plantains (*Musa* spp). In: Campos, H Caligari, P.D.S. editors genetic improvement of tropical crops. Springer International Publishing AG; 2017.
   DOI: 10.1007/978-3-319-59819-2_7
2. Swennen R, Vuylsteke D. Breeding black Sigatoka resistant plantains with a wild diploid banana. Tropical Agriculture Trinidad. 1993;70:74-78.
3. Eldridge K, Davidson L, Harwood C, Vanwyk G. Eucalypt domestication and breeding. Oxford University Press, New York. 1993;288.
4. Ortiz R. Conventional banana and plantain breeding. Acta Hoticulturae. 2013;986:1-20.
5. Moncur MW. Techniques for pollinating eucalypts. Australian Centre for International Agricultural Research, Canberra. 1995;1–19.
6. Harbard JL, Griffin AR, Espejo J. Mass controlled pollination of Eucalyptus globulus: A practical reality. Canadian Journal of Research. 1999;29:1457–1463.
7. Vuylsteke D, Swennen R, Ortiz R. Development and performance of Black Sigatoka-resistant tetraploid hybrids of plantain (*Musa* spp., AAB group). Euphytica. 1993;65:33–42.
8. Crouch HK, Crouch JH, Madsen S, Vuylsteke D, Ortiz R. Comparative analysis of phenotypic and genotypic diversity among plantain landraces (*Musa* spp., AAB group). Theor. Appl. Genet. 2000;101: 1056–1065.
9. Tripathi JN, Muwonge A, Tripathi L. Efficient regeneration and transformation of plantain cv. 'Gonja manjaya' (*Musa* spp. AAB) using embryogenic cell suspensions. *In Vitro* Cell Dev Biol. 2012;48:216–224.
10. Nyine M, Uwimana B, Swennen R, Batte M, Brown A, Hřibová E, Doležel J. Genomic breeding approaches for East African Bananas. In: Abstracts of the plant and animal genome conference XXIV January 08–13, San Diego, CA; 2016
11. Silva PRO, De Jesus On, Bragança CAD, Haddad F, Amorim EP, Ferreira CF. Development of a thematic collection of *Musa* spp accessions using SCAR markers for preventive breeding against Fusarium oxysporum f. sp cubense tropical race 4. Genet Mol Res. 2016;15: 501776.
    DOI:http://dx.doi.org/10.4238/gmr.15(1)017765

12. Noumbissié GB, Chabannes M, Bakry F, Ricci S, Cardi C, Njembele JC, Yohoume D, Tomekpe K, Iskra-Caruana ML, d'Hont A. Chromosome segregation in an allotetraploid banana hybrid (AAAB) suggests a translocation between the A and B genomes and results in eBSV-free offsprings. Mol Breed. 2016; 36:1–14.
13. Fortescue JA, Turner DW. Reproductive biology. In: Pillay M, Tenkouano A (eds) Banana breeding: constraints and progress. CRC Press, Boca Raton. 2011;305–331.
14. Tenkouano A, Ortiz R, Vuylsteke D. Estimating genetic effects in maternal and paternal half-sibs from tetraploid-diploid crosses in *Musa* spp. Euphytica 2012; 185:295–301.
15. Wairegi LWI, Van Asten, PJ, Tenywa A, Bekunda M. Abiotic constraints override biotic constraints in East African highland banana systems. Field Crop Res. 2010; 117:146–153.
16. Van Asten PJA, Fermont AM, Taulya G. Drought is a major yield loss factor for rainfed East African highland banana. Agric Water Manag. 2011;98:541–552
17. Kumar LP, Selvarajan R, Iskra-Caruana M, Chabannes M, Hanna R. Biology, etiology, and control of virus diseases of banana and plantain. In: Loebenstein G, Katis NI (eds) Advances in virus research. Academic, Burlington. 2015;91; 229–269.
18. Ortiz R. Plant breeding in the omics era. Springer, New York; 2015.
19. Umber M, Pichaut J, Farinas B, Laboureau N, Janzac B, Plaisir-Pineau K, Pressat G, Baurens F, Chabannes M, Duroy P, Guiougou C, Delos J, Jenny C, Iskra-Caruana M, Salmon F, Teycheney P. Marker-assisted breeding of *Musa balbisiana* genitors devoid of infectious endogenous banana streak virus sequences. Mol Breed. 2016;36:1–11.
DOI:10.1007/s11032-016-0493-8
20. Ortiz R. Genetic and phenotypic correlations in plantain-banana euploid hybrids. Plant Breed. 1997d;116:487–491.
21. Ortiz R. Morphological variation in *Musa* germplasm. Genet. Resour. Crop Evolution. 1997a;44:393–404.
22. Ortiz R, Vuylsteke D. Quantitative variation and phenotypic correlations in banana and plantain. Scientia Horticulturae. 1998a;72: 239–253.
23. Aguilar-Morán JF. Improvement of Cavendish banana cultivars through conventional breeding. Acta Hortic. 2013; 986:205–208.
24. Tenkouano A, Vuylsteke D, Okoro J, Makumbi D, Swennen R, Ortiz R. Diploid banana hybrids TMB2x5105-1 and TMB2x9128-3 with good combining ability, resistance to black Sigatoka and nematodes. Hort Science. 2003;38:468–472.
25. Krishnamoorthy V, Kumar N. Preliminary evaluation of diploid banana hybrids for yield potential, male fertility and reaction to *Radopholus similis*. Plant Gen Res Newsl. 2005;141:39–43.
26. Nyine M, Uwimana B, Swennen R, Batte M, Brown A, Christelova ÂP. Trait variation and genetic diversity in a banana genomic selection training population. Plos One. 2017;12(6):1-23.
27. Ortiz R, Swennen R. Review: From crossbreeding to biotechnology-facilitated improvement of banana and plantain. Biotechnology Advances. 2014;32:158-169.
28. Khan SA, Ahmad H, Khan A, Saeed M. Khan SM, Ahmad B. Using line x tester analysis for earliness and plant height traits in sunflower. Journal of Plant Breeding and Genetics. 2009;01(03):117-129.
29. Acquaah G. Principles of plant genetics and breeding. 2nd ed. Wiley-Blackwell, Oxford; 2012.
30. Abass HG, Mahmood A Ali, Saif-ul-Malook Q, Waseem M, Khan NH. Genetic variability for yield, its components and quality traits in upland cotton (*Gossypium hirsutum* L.) Nature and Science. 2014;12: 31-35.
31. Moran GF, Bell JC. Eucalyptus. In: Isozymes in plant genetics and breeding. Tanksley S.D, and Orton T. J (eds); Part B. Elsevier, Amsterdam. 1983;423–441.
32. Potts B, Potts W, Cauvin B. Inbreeding and interspecific hybridisation in *Eucalyptus gunnii*. Silvae Genetical. 1987;36(5-6):194-199.
33. Potts BM, McGowen MH, Williams DR, Suitor S, Jones TH, Gore PL, Vaillancourt RE. Advances in reproductive biology and seed production systems of Eucalyptus: The case of *Eucalyptus globulus*. South Forests. 2008;70(2):145–154.
34. Chaix G, Gerber S, Razafimaharo V, Vigneron P, Verhaegen D, Hamon S. Gene flow estimation with microsatellites in a Malagasy seed orchard of *Eucalyptus*

grandis. Theoretical and Applied Genetics. 2003;107:705–712.

35. Jones ME, Shepherd M, Henry R, Delves A. Pollen flow in *Eucalyptus grandis* determined by paternity analysis using microsatellite markers. Tree Genetics & Genomes. 2008;4(1):37-47.
36. Horsley TN, Johnson SD, Myburg AA. Comparison of different control-pollination techniques for small-flowered eucalypts. New Forests. 2010;39:75–88.
37. Tenkouano A, Baiyeri KP, Ortiz R. Phenotypic and genetic correlations in *Musa* populations in Nigeria. African Crop Science Journal. 2002;10:121–132.
38. Ortiz R, Vuylsteke D, Ferris RSB, Okoro JU, Guessan N, Hemeng A, Yeboah OB, Afreh-Nuamah K. Developing new plantain varieties for Africa. Plant Varieties and Seeds. 1997;10:39 –57.
39. Ortiz, R. Plot techniques for assessment of bunch weight in banana trials under two systems of crop management. Agronomy Journal. 1995;87:63–69.
40. Tenkouano A, Oselebe H, Ortiz R. Selection efficiency in *Musa* L. under different cropping systems. Australian Journal of Crop Science. 2010;4:74–80.
41. Swennen R. Plantain cultivation under West African conditions: A reference manual. International Institute of Tropical Agriculture, Ibadan, Nigeria. 1990;24.
42. Ortiz R, Vuylsteke D. Inheritance of dwarfism in plantain (*Musa* SPP., AAB group). Plant Breeding. 1995;114:466-468.
43. SAS Institute. SAS Users' Guide, Release 6.03 Edition Statistical Analysis Systems Institute Inc., Cary, North Carolina, U.S.A; 1992.
44. Ortiz R, Crouch JH. The efficiency of natural and artificial pollinators in plantain (*Musa* spp. AAB group) hybridization and seed production. Annals of Botany. 1997; 80:693–695.
45. Craenen K, Ortiz R. Effect of the black sigatoka resistance gene *bs1* and ploidy levels in fruit and bunch traits of plantain-banana hybrids. Euphytica. 1996;87:97–101.
46. Ortiz R. Secondary polyploids, heterosis and evolutionary crop breeding for further improvement of the plantain and banana genome. Theoretical & Applied Genetics. 1997c;94:1113–1120.
47. Bradshaw JE, Mackay GR. Breeding strategies for clonally propagated potatoes. In: J.E. Bradshaw and G.R. Mackay (ed.) Potato genetics. CAB International, Wallingford, UK. 1994;467-497.
48. Bradshaw JE. Breeding potato as a major staple crop. In: M.S. Kang and P.M. Priyadarshan (ed.) Breeding major food staples. Blackwell Publishing professional, Ames, Iowa, USA. 2007;277-332.

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