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Early Withering of Enlarged Ovules in Pollinated Fruits of Bananas (*Musa* spp.) Suggest Abortion after Fertilization

Allan Waniale [1,2], Settumba B. Mukasa [1], Arthur K. Tugume [3], Jerome Kubiriba [2], Wilberforce K. Tushemereirwe [2] and Robooni Tumuhimbise [4,*]

[1] Department of Agricultural Production, College of Agricultural and Environmental Sciences, Makerere University, Kampala P.O. Box 7062, Uganda; allanwaniale@gmail.com (A.W.); sbmukasa@caes.mak.ac.ug (S.B.M.)
[2] National Agricultural Research Laboratories, Kawanda, Kampala P.O. Box 7065, Uganda; jkubiriba2012@gmail.com (J.K.); tkwilberforce@gmail.com (W.K.T.)
[3] Department of Plant Sciences, Microbiology, and Biotechnology, College of Natural Sciences, Makerere University, Kampala P.O. Box 7062, Uganda; arthur.tugume@mak.ac.ug
[4] Rwebitaba Zonal Agricultural Research and Development Institute, Fort Portal P.O. Box 96, Uganda
* Correspondence: rtumuhimbise@hotmail.com

Abstract: Sterility in edible bananas is as a result of a long history of anthropogenic-driven selection for sterile genotypes, since seed is not desirable in fruit pulp for human consumption. However, this poses a challenge to conventional genetic improvement by slowing breeding pipelines. In this study, we investigated whether pollen tubes reach all parts of the ovary, the position of fertilized ovule development in fruits, and potential seed set in selected banana genotypes. We selected four cultivars of East African Highland Cooking bananas (EAHBs), a Matooke hybrid '222K-1', improved diploid '2905', and wild bananas 'Zebrina (G.F.)' and 'Calcutta 4'. There was evidence of pollen tubes in the distal, mid and proximal sections of the fruit, irrespective of hand position and genotype. Fertilization, as indicated by an increase in ovule size, happened along the entire length of the fruit but complete development was biased at the distal end in some genotypes. There were some differences in ovule fertilization rates between hands, with distal hands having more ovules and higher ovule fertilization rates. Ovule fertilization happens in bananas but the vast majority aborts, especially at the proximal end of the ovary. Ovule fertilization rates are generally much lower than available ovules.

Keywords: banana pollination; pollen tubes; ovule fertilization; ovule abortion

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1. Introduction

A majority of bananas and plantain (*Musa* spp.) are triploid and mostly sterile, with fruits developing parthenocarpically without the need for pollination and seed formation. They originated from natural inter- and intra-specific hybridization events between progenitors *Musa acuminata* and *M. balbisiana*, belonging to two genomic groups, AA and BB, respectively [1]. These two banana progenitors are seeded; thus, humans were involved in the selection of edible genotypes [2]. However, fertility was not totally lost, as edible diploids gave rise to triploids. They arose due to meiotic instability, which resulted in unreduced gametes (2n) that were fertilized by n gametes [3]. There were also observations of efficient pollination of artificially created tetraploids by natural pollinators, including bats, honeybees, and birds [4]. Therefore, inferences can be made on tetraploid involvement in giving rise to triploids rather than diploids only [5]. Hybridization and effective selection produced cultivars that became mostly sterile and, thus, could not easily be improved through conventional means.

Banana conventional genetic improvement programs depend on hand pollination to generate seeds but there is limited success on landraces that have residual seed fertility [6]. The banana inflorescence bears 1–30 female flower clusters, 0–4 neutral flower clusters, and

up to 300 male flower clusters [7]. The inflorescence and flower clusters are also known as the bunch and hands, respectively, while ovaries are also known as fingers. Seed set per bunch is merely a small fraction of the potential that could be obtained. Ovules per fruit are estimated to be between 300 and 1500, arranged in two or four rows in each locule [7]. In Matooke bananas, there is an average of 303 ovules per fruit, yet observed maximum seed set per bunch is 227 [8]; Batte et al. observed 305 [9]. In 'Gros Michel' crosses, Shepherd [10] recorded maximum seed set per bunch of 60, whereas 219 seeds per bunch were observed in plantain crosses [11]. Besides poor seed set in edible bananas, there are some unique patterns of seed set. One of the peculiar observations is the bias of seed set in the middle hands of the bunch [12–14], as well as a bias towards the stylar end of the fruit [10,14]. Ssebuliba et al. [13] also found a correlation of stigma receptivity with seed set in Matooke. They observed increasing stigma receptivity with increasing seed set from proximal to distal hands of the bunch.

In Matooke, different cultivars display different stigma developmental stages at the time flowers are considered ready for pollination [13]. The stigmas gradually become more receptive with time before eventually becoming senescent. It is not clear how long the stigma may remain receptive for pollen germination. Understanding all these observations may reveal the underlying factors for sterility in bananas. This study, therefore, investigated whether pollen tubes reach all sections of the ovaries of different hand positions and the section in which ovules develop. Furthermore, the pollination period and influence of temperature on ovule fertilization rates, as well as total ovules per fruit, were investigated. This article is a contribution to the knowledge of the most important factors that contribute to sterility in bananas.

2. Materials and Methods

2.1. Study Site and Banana Genotypes Used

The study was conducted in Uganda at the National Agricultural Research Laboratories (NARL) in Kawanda located at 0°25′ N and 32°32′ E at an elevation of 1177 m. Banana genotypes used in the study included *Musa* (AA group subgroup Mchare) 'Mshale' and 'Mlelembo' as well as *Musa* (AAA-EA group, Matooke subgroup) 'Enzirabahima' and 'Nakitembe'. Matooke and Mchare banana subgroups are endemic to the East African region [15]; they can be collectively referred to as East African Highland Cooking bananas (EAHBs). Wild bananas (*Musa acuminata* ssp. *burmannicoides* 'Calcutta 4' (AA) and 'Zebrina (G.F.)' (AA)), improved diploid '2905' (AA), and the hybrid Matooke '222K-1' (AAAA) were also included in the study. Hybrid '222K-1' is a result from cross between *Musa* (AAA-EA group, Matooke subgroup) 'Nfuuka' and 'Calcutta 4'; it is used in breeding of Matooke as a female parent. 'Zebrina (G.F.)' and improved diploid '2905' are used as male parents in the Matooke conventional improvement program at NARL. All pollinations in this study were made with 'Calcutta 4' as the pollen source.

2.2. Ascertaining Pollen Tube Reach in Ovaries

Artificial pollinations were made on 'Calcutta 4' bunch hand 6, 'Mshale' bunch hand 3, 'Mlelembo' bunch hand 6, 'Enzirabahima' bunch hand 1, and 'Nakitembe' bunch hand 3. The pollination technique was performed as described by Vuylsteke et al. [16] but modified by spraying a fine mist of liquid pollen germination media (PGM) on pollen that had been dusted on stigmas to enhance receptivity [17]. Preparation of samples for pollen tube viewing was according to the Thompson and Mitchell protocol [18]. Samples of three ovaries for each genotype were randomly selected from hands on the bunch 48 h after pollination. Banana pollen tube growth rate in the style is about 0.33 mm/h [7]. In Matooke, style length averages 2.80 cm while ovaries average 11.68 cm [8]. The total combined average length of style and ovaries is therefore 14.48 cm. Pollen tubes are therefore expected to traverse the styles and ovaries within 44 h. This is the rationale of sampling 48 h after pollination. The ovaries were peeled to expose ovules on the placenta and were immediately fixed in farmer's solution for a minimum of 24 h. Farmer's solution

was prepared using 3 parts ethyl alcohol and 1 part acetic acid. The peeled ovaries were then washed in distilled water for 10 min and softened in a 10 M NaOH solution for two weeks; the solution was refreshed after one week. Softening softens the tissues and makes them more receptive to the dyes. The Thompson and Mitchell protocol recommended 4–24 h softening time for genus *Mimulus* but a longer period was used for banana as samples were bigger. The samples were occasionally shaken gently to mix the layered solution.

After softening, samples were rinsed for 5 min twice in 0.5 M KH_2PO_5 solution and once in 0.1% aniline blue in 0.5 M KH_2PO_5, as this ensures a better response to the stain. The samples were then decolorized using 0.1% aniline blue in 0.5 M KH_2PO_5 for two hours in full darkness [18]. Sections of the ovary from the distal, middle and proximal parts were placed on glass slides and rows of ovules separated with a needle. One or two drops of glycerol were put on the sections of ovules before gently squashing between two glass slides. The samples were viewed under UV light with a Nikon Eclipse TS100-F fluorescence microscope. The experiment was performed in May 2019.

2.3. Determining the Position of Fertilized Ovule Development in Ovaries

One bunch each of diploids 'Zebrina GF' and '2905', Matooke cultivars 'Nakitembe' and 'Enzirabahima', and Matooke hybrid '222K-1' were pollinated as described in Section 2.2. On the same bunch, all flowers in odd hand positions were artificially pollinated while flowers in even hand positions were unpollinated as controls. Ovaries were longitudinally split open at intervals of 10–12 days and 20–22 days after pollination. Ovule development was examined and photographs taken with a Nikon Coolpix D850 digital camera. Both pollinated and unpollinated ovaries were examined. The experiment was performed in September 2019.

2.4. Evaluation of the Pollination Period and Potential Seed Set

Pollinations were made a day before anthesis (day −1), the day of anthesis (day 0), and at day +1, +2, and +3 after anthesis. In each selected hand on the bunch, three flowers were pollinated for each earmarked day after anthesis (DAA) until all timings were covered. Besides the control artificial pollination, the second technique was a modification by spraying a fine mist of liquid pollen germination media (PGM) with pollen on stigmas to enhance receptivity [17] (the +PGM technique). Three bunches each with the two pollination techniques were performed on 'Calcutta 4', 'Mlelembo', 'Mshale', 'Enzirabahima', and 'Nakitembe' on hand positions 1, 3, and 5 of the bunch. Artificial pollinations were made between May and September 2019. To assess the effective pollination period, ovaries were picked and split open to expose ovules between 14 and 21 days after artificial pollination. Ovules that distinctly increased in size were presumed to have been fertilized and those that remained small were presumed to be unfertilized. The two categories were counted and recorded separately. Increase in ovule size as an indicator of fertilized ovules is as used in seedless grapes [19]. Ovule fertilization rates were also assessed in open pollinated 'Calcutta 4' by sampling three ovaries from hands 1, 3, and 5 of three bunches.

Percentage fertilization rate was calculated as number of ovules that visibly grew large over total ovules multiplied by 100. Percentage fertilization rates of three ovaries for each day of flower opening on different hands, bunch, genotype, and pollination technique were averaged. Analysis of variance (ANOVA) was performed to compare ovule fertilization rates between pollination techniques, hand position and DAA for each genotype. For 'Calcutta 4', the two hand pollination techniques were also separately compared with open pollination in the ANOVA. In all analyses, bunches were treated as replicates while hand positions and DAA were treated as treatment factors. To get potential seed set per fruit, large and small ovules were added as per the plan of measuring the pollination period. For each genotype, number of ovules per ovary was compared in bunch hands 1, 3, and 5 while ignoring DAA and averages of three fruits. All analyses were performed in Genstat 19th edition developed by VSN International (VSNi).

2.5. Temperature Influence on Ovule Fertilization Rates

Daily temperature data taken at 15:00 h were obtained from the NARL agro-metrological station. The temperature range on study days was 24.5–30.8 °C with an average of 27.8 °C. A Pearson's correlation was conducted between temperature and ovule fertilization rates on different days. Correlation was conducted separately for different genotype and pollination techniques as well as combined data under different pollination techniques. The analyses were performed with Genstat 19th edition software developed by VSNi.

3. Results

3.1. Pollen Tube Observations

There was evidence of pollen tubes in all parts of the ovary 48 h after pollination and after staining with aniline blue. However, the pollen tubes were covered by excessive plant tissue and clear images could not be captured; quantification was also impossible. In 'Calcutta 4', pollen tubes were found in the proximal, middle, and distal sections of the ovary. The ovaries were sampled from bunch hand 6. In 'Mshale', pollen tubes were in the distal parts of the ovary sampled from bunch hand 3. Pollen tubes were also observed to have reached ovules in ovaries taken from the first hand of the seed fertile 'Enzirabahima' and bunch hand 6 of the seed sterile 'Mlelembo'.

3.2. Position of Developing Ovules

Ovules in unpollinated fruits withered, while in pollinated fruits, some ovules grew in size, depending on genotype and days after pollination. In 'Nakitembe', ovules had withered by 10 days after pollination (DAP) in unpollinated fruits. On the other hand, in pollinated fruits, some ovules presumed to be fertilized remained fresh while those presumed to be unfertilized withered 10 DAP (Figure 1A). For diploid 'Zebrina (G.F.)', there was no noticeable difference between ovules from pollinated and unpollinated fruits 11 DAP (Figure 1B). For tetraploid hybrid Matooke '222K-1', there was an early and uniform withering of all ovules in unpollinated fruits, whereas some ovules developed in pollinated fruits 12 DAP (Figure 1C).

Ovules from unpollinated '2905' fruits did not increase in size and had withered uniformly by 20 DAP (Figure 1D). In pollinated fruits, a few ovules continued to develop, irrespective of their position on the placenta; there was also pulp development. There was a distinct necrosis in the prolongation zone of pollinated fruits but not in unpollinated fruits (Figure 1D). At 20 DAP, some ovules in unpollinated fruits of 'Zebrina (G.F.)' were still fresh, although some had started withering. In pollinated fruits, some ovules had noticeably increased in size; some remained small but fresh, while others had withered. The largest ovules in pollinated fruits were those presumed to be fertilized while the rest were presumed to be unfertilized 20 DAP (Figure 1E). At 22 DAP, there was a bias of ovule development at the stylar end of Matooke hybrid '222K-1' fruits. Larger and presumably fertilized ovules in the midsection and at the proximal fruit end had withered by 22 DAP (Figure 1F).

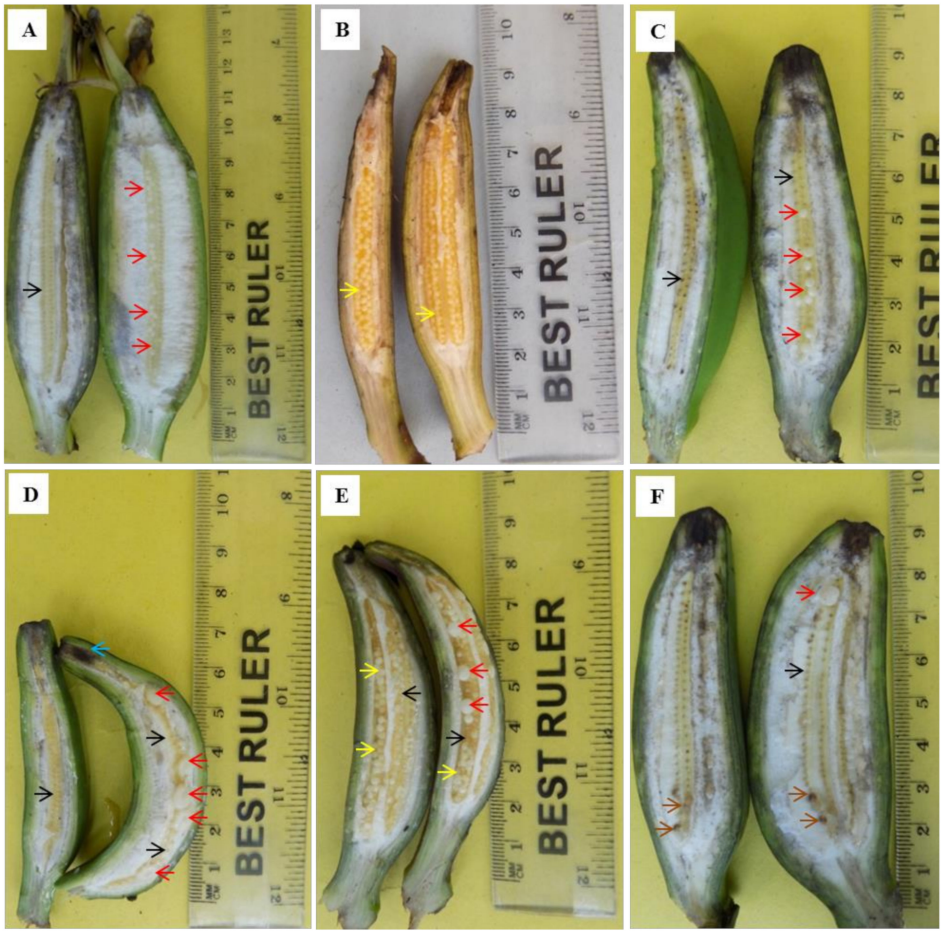

Figure 1. Ovule development stages in selected banana genotypes from pollinated and unpollinated fruits. With the exception of (**F**) with both fruits pollinated, unpollinated fruits are on the left and pollinated fruits on the right. Black arrows—withering ovules; red arrows—developing ovules; yellow arrows—fresh ovules; brown arrows—presumably fertilized but withered ovules; blue arrow—necrosis in prolongation zone. (**A**) 'Nakitembe' 10 DAP. (**B**) 'Zebrina (G.F.)' 11 DAP. (**C**) '222K-1' 12 DAP. (**D**) '2905' 20 DAP. (**E**) 'Zebrina (G.F.)' 20 DAP. (**F**) '222K-1' 22 DAP.

3.3. Ovule Fertilization Rates

The average ovule fertilization rates after pollination with and without PGM were significantly different in all five banana genotypes, except in 'Mshale' (Table 1). The control pollination technique gave significantly higher fertilization rates compared to the pollination with PGM in 'Calcutta 4' and 'Mlelembo'. 'Mlelembo', 'Enzirabahima', and 'Nakitembe' had significantly higher ovule fertilization success rates in hand 5 position after pollination with the control technique. 'Mshale' and 'Enzirabahima' showed significant differences in ovule fertilization rates of different hand positions after pollinating with PGM. There were significantly higher ovule fertilization rates in distal hands of 'Enzirabahima', while the proximal hands of 'Mshale' had a higher mean after pollination with PGM. Only 'Nakitembe' had a significant difference in ovule fertilization rates after control pollination on different DAA, and its highest mean was after pollinating at 2 DAA. There were no significant differences in ovule fertilization rates after pollination with PGM on different DAA in the five genotypes (data not presented).

Table 1. Mean percentage fertilized ovules after pollination on different days after anthesis and in different hands in selected banana genotypes.

Technique	Hand	'Calcutta 4'	'Mlelembo'	'Mshale'	'Enzirabahima'	'Nakitembe'
Control	1	40.1	9.9 b	10.2	6.6 b	2.9 b
	3	41.8	16.0 a	8.4	6.9 ab	3.8 a
	5	40.8	17.6 a	8.2	7.4 a	3.9 a
LSD		2.9	4.3	3.9	0.6	0.4
p value		0.526	0.002	0.570	0.028	<0.001
+PGM	1	36.7	7.9	9.6 a	7.1 b	8.2
	3	37.8	7.5	8.5 b	8.1 a	7.7
	5	34.8	8.4	8.6 ab	8.5 a	5.8
LSD		2.6	0.8	0.8	0.6	2.0
p value		0.069	0.066	0.024	<0.001	0.060
	DAA					
Control	−1	42.5	12.7	8.2	7.3	3.0 b
	0	40.4	15.9	12.3	6.9	3.5 ab
	1	43.3	15.9	8.4	6.9	3.4 ab
	2	40.5	15.1	8.4	7.1	3.9 a
	3	38.0	13.1	8.1	6.6	3.8 ab
LSD		3.8	5.5	4.9	0.8	0.6
p value		0.068	0.639	0.361	0.715	0.026
Mean—Control		40.9	14.5	9.4	7.0	3.5
Mean—+PGM		36.4	7.9	8.8	7.9	7.3
p value		0.005	0.017	0.290	<0.001	<0.001

Means with different letters in different columns are statistically different, LSD—least significant difference.

The highest numerical average ovule fertilization rate in 'Calcutta 4' for controlled pollination on different DAA was 1 DAA (Table 1). After comparing means of control and pollination with PGM at 1 DAA with mean of open pollination, there was a significant difference ($p < 0.001$). Open pollination had 62.6% ovule fertilization success rate, which was significantly different from control pollination at 43.3% and pollination with PGM at 36.6%. Ovule fertilization rates in different hand positions were not significant.

3.4. Ovules per Fruit

There were significant differences in number of ovules per fruit in different hand positions in all genotypes except for 'Mshale' (Table 2). Bunch hands in position 1 had fruits with the lowest number of ovules, which increased towards the distal end of the bunch. There were also significant differences in mean ovules per fruit among selected banana genotypes.

Table 2. Mean number of ovules per fruit in different hands among selected banana genotypes.

Hand	'Calcutta 4'	'Mlelembo'	'Mshale'	'Enzirabahima'	'Nakitembe'	Mean
1	214.5 a	191.0 a	377.3 a	355.7 a	282.6 a	269.1 a
3	219.0 ab	198.9 ab	382.7 a	365.1 b	303.7 b	278.1 ab
5	233.7 b	205.6 b	378.0 a	375.4 c	311.5 b	282.4 b
Mean	222.4 B	198.5 A	379.3 E	365.4 D	299.3 C	

Means with different letters are statistically different, lower-case letters compare means of hand positions of the same genotype and overall hand position means, upper-case letters compare genotypes.

3.5. Influence of Temperature on Ovule Fertilization Rates

There was no significant relationship between temperature and ovule fertilization for pollination with or without PGM (Table A1). Indeed, the R^2 values for each selected banana genotype after pollination with and without PGM were very low.

4. Discussion

In banana breeding, it is a common practice to apply more than enough pollen to fertilize all ovules [10]. There is also ample pollen germination on the stigma [17] but there appears to be pollen tube arrest in the style before they reach ovules [10]. Pollen tube arrest is also believed to happen as a result of a physical barrier of necrosis in the prolongation zone, especially in triploids but not in diploids [20]. Pollination of 'Mshale', 'Mlelembo', 'Enzirabahima', and 'Nakitembe' showed that 'Mshale' and 'Enzirabahima' are seed fertile while 'Mlelembo' and 'Nakitembe' are seed sterile [21]. In the current study, pollen tubes were observed in the sterile cultivar 'Mlelembo', which rules out the absence of pollen tubes as a major contributor to sterility.

Clearly, not all pollen tubes are arrested as some find their way into the ovary. There is a strong bias of seed set in the midsection hands [12–14] and towards the distal ends of the fruits [10,14]. However, since there were pollen tubes in the midsection of 'Enzirabahima' fruits sampled from the first hand, pollen tube growth does not account for these seed set patterns. Caution has to be taken in drawing conclusions from this part of the study, since one hand from one bunch was pollinated. Besides, not all hands were sampled for the genotypes that were studied for pollen tube evidence study. It also has to be noted that the protocol for pollen tube observation [18] was not very suitable for banana, as it needs further improvement.

With certainty that pollen tubes reach the ovules in any part of the ovary, ovule development was observed in some pollinated fruits, suggesting successful fertilization. Uniform withering of ovules suggested no fertilization, whereas differential withering in pollinated fruits suggested some fertilization. In seedless grapes, fertilized ovules are also observed to increase in size [19]. For the fertile diploid 'Zebrina (G.F.)', the results suggest that ovules take longer to disintegrate as there are no noticeable differences in pollinated and unpollinated fruits 11 DAP. In the tetraploid Matooke '222K-1', the continuation of ovule development became biased towards the stylar end of the ovary. The ovules that developed to a bigger size than the rest in 'Nakitembe' eventually aborted.

Counting ovules that slightly developed after pollination was, therefore, a good way to evaluate ovule fertilization rates in 'Calcutta 4' and selected EAHBs. It was expected that pollination with PGM would result in higher ovule fertilization rates but results were inconsistent. This partly came as a result of making pollinations on different days and different months, thus, it could be a season effect [21]. Significantly higher fertilization rates in Matooke after pollination with PGM implies that stigma receptivity enhancing in Matooke is much more beneficial than pollination without enhancing stigma receptivity [21]. The results suggest that pollinations of Matooke can be made from about a day before flower opening up to three DAA. This suggests that on days of pollen shortage, pollination can be made on subsequent days. These results also imply that repeated pollinations on different days can be made to ensure maximum fertilization rates of ovules. However, validation studies have to be conducted to ascertain seed set after pollination on different DAA.

Increasing stigma receptivity from proximal to distal hands in Matooke, which correlated with seed set, was reported [13]. After stigma receptivity was enhanced with PGM, differences in ovule fertilization rates from hand 1 to hand 5 were not expected. However, 'Enzirabahima' had more ovule fertilization rates in distal hands than proximal hands. It is unclear why the first hand of 'Mshale' had the higher mean than in latter hands, especially after pollination with PGM. The pattern observed in 'Enzirabahima' implied that PGM was not enough to even out ovule fertilization rates between hands. The likely cause of this observation is having more pollen tube arrest in proximal hands compared to distal hands of 'Enzirabahima'. There are also significantly more ovules in hand 5 position compared to hand 1 position in the bunch. This may partly explain why converting seed set to 100 fruits basis per hand still revealed that more seeds set in distal hands [14]. Ovules per fruit also differ between genotypes; thus, comparison of seed set between hands and between genotypes should, therefore, be on a seed set per specific unit number of ovules.

As expected, 'Calcutta 4' had the same ovule fertilization rates in all hands, whether pollinated with or without PGM, as it is highly fertile. However, ironically, open pollination had significantly higher success rates compared to artificial pollinations. Further, pollination with PGM had significantly less means than control pollination technique yet there was greater and quicker germination of pollen with the former technique [17]. This suggests a self-incompatibility system in *Musa* spp., since self-pollinations had less means. Simmonds [22] also reported more seed yield in crossing closely related clones on *M. acuminata* and those of *M. balbisiana* than their self-pollinations. A mixture of pollen sources and gradual repeated pollination may explain the higher success rate in open pollination of 'Calcutta 4', since the effective pollination period is wide. Using a mixture of compatible and incompatible pollen has been reported to overcome gametophytic self-incompatibility in some plant species [23,24]. Triggering of more and quick pollen germination after pollination with PGM implies that many pollen tubes simultaneously grow down in the style, and this may trigger a stronger self-incompatibility response, thus, less seed. However, this seems to be "partial self-incompatibility", since there is ovule development in 'Calcutta 4' self-pollinations.

High temperature, high solar radiation, low rainfall amounts and low relative humidity (RH) have been observed to increase seed set when banana flowers are artificially pollinated [25,26]. A relationship between temperature and ovule fertilization rates was, therefore, expected. However, based on correlation coefficients and R^2, it can be said that temperature does not have any effect on ovule fertilization rates under highland conditions. This suggests that pollen tube growth is delayed but pollen tubes eventually reach the ovules. It also suggests that late arrival of pollen tubes result in fertilization but the ovules abort, and disintegration of ovules is thought to start 24 h after anthesis [7].

5. Conclusions

In edible bananas with residual fertility, there is always a bias of seed set towards the stylar end of the fruit and in distal hands of the bunch. The underlying causes of these observations have not been clear. Answering these questions contributes to the body of knowledge that will ultimately overcome sterility in bananas. The study investigated pollen tube evidence in the ovaries of bananas, ovule development patterns, the pollination period, potential seed set, as well as the relationship between temperature and ovule fertilization rates. Pollen tubes were able to reach all parts of the ovary, irrespective of the hand position of the bunch in both sterile and fertile cultivars. Consequently, there seemed to have been profuse ovule fertilization but the vast number of ovules abort development in most genotypes. In the most sterile edible genotypes, presumably fertilized ovules abort sooner. Conversely, genotypes with residual-to-high fertility have considerable ovule development before abortion. In these genotypes, seed development was confirmed to be biased towards the stylar end of the fruit. The study also confirmed that pollinations made one day before anthesis up to three days after anthesis lead to successful fertilization. Efforts to increase seed set should, therefore, focus on ensuring that fertilized ovules develop into seed followed by increasing ovule fertilization rates. The temperature during pollination seems to have no effect on ovule fertilization.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Summary statistics for temperature (°C) and percentage fertilized ovules in five banana genotypes after pollination with 'Calcutta 4' pollen from May to September 2019.

Genotype	Control Pollination				PGM Pollination			
	r	Prob	R^2	n	r	Prob	R^2	n
'Calcutta 4'	0.055	0.718	0.003	45	0.350	0.002	0.123	75
'Mlelembo'	−0.100	0.515	0.010	45	0.228	0.083	0.052	59
'Mshale'	0.150	0.364	0.022	39	−0.030	0.881	0.001	28
'Enzirabahima'	0.080	0.615	0.006	42	−0.170	0.289	0.029	41
'Nakitembe'	0.113	0.472	0.013	43	0.144	0.475	0.021	27
All	0.024	0.731	0.001	214	0.242	<0.001	0.059	230

r—correlation coefficient, Prob—Two-sided test of correlations different from zero, R^2—R-*squared* which is a goodness-of-fit measure for linear regression models, n—number of observations, Control—Pollination as described by [16], PGM—pollination with pollen germination media [17].

References

1. Heslop-Harrison, J.S.; Schwarzacher, T. Domestication, Genomics and the Future for Banana. *Ann. Bot.* **2007**, *100*, 1073–1084. [CrossRef] [PubMed]
2. Subbaraya, U.; Saraswathi, M.S.; Pillay, M. Evolution and Genetic Relationships in Banana and Plantains. In *Banana Breeding: Progress and Challenges*; Pillay, M., Tenkouano, A., Eds.; CRC Press: Boca Raton, FL, USA, 2011. [CrossRef]
3. Perrier, X.; Bakry, F.; Carreel, F.; Jenny, C.; Horry, J.-P.; Lebot, V.; Isabelle, H. Combining biological approaches to shed light on the evolution of edible bananas. *Ethnobot. Res. Appl.* **2009**, *7*, 199–216. [CrossRef]
4. Mutsaers, M. Natural pollination of plantain and banana at Onne. *MusAfrica* **1993**, *2*, 2–3.
5. Ortiz, R.; Crouch, J.H. The efficiency of natural and artificial pollinators in plantain (*Musa* spp. AAB group) hybridization and seed production. *Ann. Bot.* **1997**, *80*, 693–695. [CrossRef]
6. Tenkouano, A.; Pillay, M.; Ortiz, R. Breeding Techniques. In *Banana Breeding: Progress and Challenges*; Pillay, M., Tenkouano, A., Eds.; CRS Press: Boca Raton, FL, USA, 2011. [CrossRef]
7. Fortescue, J.A.; Turner, D.W. Reproductive Biology. In *Banana Breeding: Progress and Challenges*; Pillay, M., Tenkouano, A., Eds.; CRC Press: Boca Raton, FL, USA, 2011; pp. 145–179. [CrossRef]
8. Ssebuliba, R.N.; Rubaihayo, P.; Tenkouano, A.; Makumbi, D.; Talengera, D.; Magambo, M. Genetic diversity among East African Highland Bananas for female fertility. *Afr. Crop Sci. J.* **2005**, *13*, 13–26.
9. Batte, M.; Swennen, R.; Uwimana, B.; Akech, V.; Brown, A.; Tumuhimbise, R.; Hovmalm, H.P.; Geleta, M.; Ortiz, R. Crossbreeding East African Highland Bananas: Lessons Learnt Relevant to the Botany of the Crop After 21 Years of Genetic Enhancement. *Front. Plant Sci.* **2019**, *10*, 81. [CrossRef] [PubMed]
10. Shepherd, K. Seed Fertility of the Gros Michel Banana in Jamaica. *J. Hortic. Sci.* **1954**, *29*, 1–11. [CrossRef]
11. Swennen, R.; Vuylsteke, D.; De Smet, K. Season dependent seed set in plantain. *Banan. Newslett.* **1991**, *14*, 35–36.
12. Swennen, R.; Vuylsteke, D. *Aspects of Plantain Breeding at IITA*; INIBAP Workshop on Sigatoka Leaf Spot Diseases (Mycosphaerella spp.); Jose, S., Rica, C., Eds.; INIBAP: Montpellier, France, 1990; pp. 252–266.
13. Ssebuliba, R.; Magambo, M.; Talengera, D.; Makumbi, D.; Tenkouano, A.; Rubaihayo, P.; Pillay, M. Biological factors affecting seed production in East African Highland Bananas. *J. Crop Improv.* **2006**, *16*, 67–79. [CrossRef]
14. Waniale, A.; Swennen, R.; Mukasa, S.B.; Tugum, A.K.; Kubiriba, J.; Tushemereirwe, W.K.; Batte, M.; Brown, A.; Tumuhimbise, R. Seed set patterns in East African Highland Cooking bananas show asymmetric distribution in bunches and fruits. *Agronomy* **2021**, *11*, 763. [CrossRef]
15. Perrier, X.; Jenny, C.; Bakry, F.; Karamura, D.; Kitavi, M.; Dubois, C.; Hervouet, C.; Philippson, G.; De Langhe, E. East African diploid and triploid bananas: A genetic complex transported from South-East Asia. *Ann. Bot.* **2019**, *123*, 19–36. [CrossRef] [PubMed]
16. Vuylsteke, D.; Ortiz, R.; Ferris, R.S.B.; Crouch, J.H. Plantain improvement. *Plant Breed. Rev.* **1997**, *14*, 267–320.

17. Waniale, A.; Mukasa, S.B.; Tugume, A.K.; Tumuhimbise, R.; Kubiriba, J.; Swennen, R. Glucose performs better than fructose, sucrose, and diluted nectar for germination of banana pollen. *Acta Hortic.* **2020**, *1282*, 269–276. [CrossRef]
18. Thompson, C.; Mitchell, R. *Techniques for Viewing Pollen Tubes in Angiosperm Flowers*; Honors Research Projects; The University of Akron: Akron, OH, USA, 2016.
19. Gray, D.J.; Mortensen, J.A.; Benton, C.M. Ovule culture to obtain progeny from hybrid seedless bunch grapes. *J. Am. Soc. Hortic. Sci.* **1990**, *115*, 1019–1024. [CrossRef]
20. Soares, T.L.; de Souza, E.H.; de Carvalho Costa, M.A.P.; de Oliveira e Silva, S.; dos Santos-Serejo, J.A. In vivo fertilization of banana. *Cienc. Rural* **2014**, *44*, 37–42. [CrossRef]
21. Waniale, A.; Swennen, R.; Mukasa, B.; Tugume, A.K.; Kubiriba, J.; Tushemereirwe, W.K.; Amah, D.; Tumuhimbise, R. Application of pollen germination media on stigmas during pollination increases seed set in East African Highland Cooking Bananas (*Musa* spp.). *Agronomy* **2021**, *11*, 1085. [CrossRef]
22. Brown, C.R.; Adiwilaga, K.D. Use of rescue pollination to make a complex interspecific cross in potato. *Am. Potato J.* **1991**, *68*, 813–820. [CrossRef]
23. Van Tuyl, J.M.; De Jeu, M.J. Methods for overcoming interspecific crossing barriers. In *Pollen Biotechnology for Crop Production and Improvement*; Sawhney, V.K., Shivanna, K.R., Eds.; Cambridge University Press: Cambridge, UK, 1997; pp. 273–293. [CrossRef]
24. Simmonds, N.W. Experiments on the pollination of seeded diploid bananas. *J. Genet.* **1952**, *51*, 32–40. [CrossRef]
25. Ortiz, R.; Vuylsteke, D. Factors influencing seed set in triploid *Musa* spp. L. and production of euploid hybrids. *Ann. Bot.* **1995**, *75*, 151–155. [CrossRef]
26. Ssebuliba, R.; Makumbi, D.; Pillay, M. Patterns of seed set in East African Highland Banana (*Musa* spp.) hybrids. *J. New Seeds* **2009**, *10*, 160–170. [CrossRef]