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# Promising and Failed Breeding Techniques for Overcoming Sterility and Increasing Seed Set in Bananas (*Musa* spp.)

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**Abstract:** Most banana improvement programs are restricted to using a sub-set of edible landraces for sexual hybridization as the majority are female sterile. This results from an array of factors that work in tandem and lead to sterility. Use of pollen germination media (PGM) during pollination significantly increases seed set, but it is a very small fraction compared to the potential seed set. This research therefore explored early pollination (a day before anthesis), evening pollination, saline treatment, plant growth regulators (PGRs) treatment, and ovule culture as potential techniques for overcoming sterility in bananas. Early and evening pollinations did not increase seed set because of immature flowers and a mismatch of male and female flower opening, respectively (t-prob. = 0.735 and 0.884). Immersion of bunches in a saline solution before pollination and ovule culture also did not overcome pollination barriers. Auxin antagonists (TIBA and salicylic acid) increased seed set, though their respective increases were not statistically significant (t-prob. = 0.123 and 0.164, respectively). The use of auxin antagonists shows great potential for overcoming pollination barriers in bananas. However, application rates and time of application have to be optimized and used holistically with other promising techniques, such as use of PGM.

**Keywords:** banana breeding; banana sterility; pollination techniques; seed set; PGRs



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## 1. Introduction

Bananas and plantains, hereafter referred to as bananas, are the world's most important fruit crop, and they are grown in tropical and subtropical countries. They are giant perennial herbaceous plants with an all-year fruiting habit. In 2021, their global production stood at 182 million tons over an estimated 12.5 million hectares in 130 countries [1]. Of the total production of bananas, excluding plantains in 2022, only 19.1 percent was exported [2]; the rest were consumed locally. Despite the importance of bananas, and associated improvement efforts, they are still affected by both abiotic and biotic stresses. The most imminent is now Fusarium wilt caused by *Fusarium oxysporum* Schlechtend. f. sp. *Cubense* tropical race 4, which is rapidly spreading to various banana growing regions [3–5]. The conventional improving of bananas for increased yield, better consumer attributes, and resistance against stresses has been hampered by inherent male and female sterility [6,7].

More emphasis has been put on understanding the gametophyte than the sporophyte as it makes a greater contribution to low seed set [8]. Female sterility results from a complex array of factors that act collectively to produce a seedless phenotype. Meiotic errors, embryo sac defects, pollen tube growth inhibition, and pollen–pistil interactions all contribute to female sterility. Sterility is also compounded by weather involvement, whereby low temperatures during bunch development reduces seed set [9]. Enhancing stigma receptivity using pollen germination media significantly increases seed set by minimizing negative

outcomes of pollen–pistil interactions [10]. However, the increase is still far below the potential seed set [11]. At the molecular level, a genome investigation identified candidate genomic regions involved in the pathways of gibberellin, auxin, abscisic acid, and cytokinin signaling [6]. The involvement of these hormones in banana male and female sterility is not yet fully understood. There is also evidence that fertilization happens, but the vast majority of fertilized ovules abort [11]. As a result of sterility in edible bananas, total gene pools are underutilized, as some preferred landraces have been rendered "infertile" [12,13]. Overcoming sterility would therefore broaden the parental base in improvement programs.

There has never been deliberate application of plant growth regulators (PGRs) with the aim of overcoming sterility in bananas, yet they have been successfully used in other crops. Successful applications include 6-Benzylaminopurine (BAP) in wide crosses of tulips and lily crosses [14], and gibberellic acid has been shown to overcome pre-pollination barriers in *Chrysanthemum grandiflorum* (Ramat.) Kitamura and *C. nankingense* (Nakai) Tzvel. (Asteraceae) [15]. Auxins and cytokinins are also among PGRs that have been successfully used [14]. Auxins have been reported to induce parthenocarpy in bananas [16]; however, they also deter seed development [17]. Experiments to antagonize auxins may, therefore, lead to overcoming sterility. Auxins can induce parthenocarpic fruit development in tomatoes, with the process being partly mediated by gibberellins [18]. Antagonizing gibberellins could deter fruit development in bananas as a result of improper functioning of auxins. Abscisic acid is another hormone that has been implicated in early and late seed development [19]; thus, exogenous applications could potentially result in seed set in bananas.

Some of the other approaches used to overcome sterility include style pollination [20], mixed and mentor pollen techniques [21], heating of styles to inactivate heat-sensitive pollen tube inhibitors [21], and ovule cultures [22]. Techniques that have worked in other crops may not necessarily work for bananas, and modifications may be required. Hormones that surge with seasonal changes, especially conditions that favor seed set, are candidate hormones for increasing seed set. High temperature, higher solar radiation, low relative humidity, and low rainfall are conditions that have been reported to increase seed set in bananas [12,23]. The aim of this study was, therefore, to develop in vivo pollination techniques and in vitro techniques that can be adopted for sterility in East African Highland cooking bananas (EAHB). The results of this study can be extrapolated to other edible banana groups.

## 2. Materials and Methods

### *2.1. Experimental Site and Banana Genotypes Used*

This experiment 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. The banana genotypes used included *Musa* (AAA group Matooke sub-group) 'Enzirabahima', 'Mbwazirume', and 'Nakitembe'. Also used were *Musa* (AA group sub-group Mchare) 'Mshale', 'Nshonowa', and 'Mlelembo'. Matooke and Mchare banana types belong to the same genetic complex [24], and can be collectively referred to as East African Cooking Bananas. Among the Matooke landraces, 'Enzirabahima' has considerably high female fertility, while 'Mbwazirume' and 'Nakitembe' are regarded as "sterile" [23,25]. The highly fertile wild banana *Musa acuminata* subsp. *burmannicoides* De Langhe (Musaceae) 'Calcutta 4' was used as the pollen source for all pollinations. The pollination blocks were planted at a spacing of 3 m between rows and 2 m between plants in plots of 9 × 22 mats. The pollen source 'Calcutta 4' was planted at the beginning and end of female parent rows. The plant population of the pollination blocks was, therefore, 1667 plants per hectare. The pollination block was managed optimally after planting with manure and top dressing with 80 g of NPK (17:17:17) inorganic fertilizer per mat.

### 2.2. Early and Evening Pollination Techniques

The control pollination technique was hand pollination, as described by Vuylsteke et al. (1997) [26] for plantain. The emerging bunches designating the female parent were bagged a day before the start of bract opening to avoid contamination with pollen from unknown sources, normally by bats and insects [27,28]. Male buds were also bagged in such a way that a bract with fresh pollen was obtained for pollination the next day. Pollination was performed by excising a hand of male flowers and brushing against the stigmas of female flowers [23,26,28]. The early pollination technique involved forcing bracts open about a day before natural opening; the free and compound tepals were pulled aside to expose the stigmas. Pollen was then dusted on the stigmas and pollen germination media (PGM) was sprayed with a hand spray pump in a fine mist from 15–20 cm to enhance receptivity [10]. A fine mist ensured that pollen was just moist but not washed off from the stigmas. The rationale was to boost moisture and energy availability for pollen germination. The evening pollination technique involved pollination between 16:00 and 18:00 h, when female flowers had just opened [29]. Stigma receptivity was also enhanced as described for early pollination. Pollen germination media was prepared with tap water, 30 g/L glucose as a substitute for sucrose, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.25 g $KNO_3$, 0.4 g $Ca(NO_3)_2$, and boric acid at 0.1 g/L [10,30]. Each treatment was applied to individual bunches from start to finish of pollination. The two pollination techniques were applied on the landrace 'Enzirabahima'.

### 2.3. Hormonal Treatment

'Mshale', 'Mlelembo', 'Enzirabahima', 'Nakitembe', and 'Mbwazirume' were hand pollinated with 'Calcutta 4' pollination as described by Vuylsteke et al. (1997) [26], and stigma receptivity was enhanced [10]. PGRs were then sprayed with a knapsack sprayer on the bunches just a day after pollinating the last female hand. For salicylic acid, injections into the pseudo stem were also experimented with just after pollination. Some bunches were left unsprayed/untreated as the control. The different PGRs, application rates, modes of application, and periods of application are summarized in Table 1.

**Table 1.** Plant growth regulators applied for seed set increase on East African Highland cooking bananas.

| Name of PGR | Rate (ppm) | Method of Application | Period |
|---|---|---|---|
| B-Nine, gibberellic acid (GA) inhibitor | 5000 (recommended rate) | Foliar, freshly pollinated fruits | Marchto September 2018 |
| Abscisic acid (ABA), plant hormone | 500 and 1000 | Foliar, freshly pollinated fruits | March to September 2018 |
| 6-benzylamino purine (6BAP), cytokinin | 500, 1000, and 2000 | Foliar, freshly pollinated fruits | August to December 2018 |
| Thiourea | 1000, 7612, 15,224, and 38,060 | Foliar, freshly pollinated fruits | May to June 2018 |
| Triiodo benzoic acid (TIBA), auxin inhibitor | 500 and 1000 | Foliar, freshly pollinated fruits | May to June 2018 |
| Salicylic acid (SA), plant hormone | 100 and 200 | Injection, foliar, freshly pollinated fruits | December 2017 to January 2018 |

PGR, plant growth regulator; rate (ppm), application rate measured in parts per million.

### 2.4. Saline Solution Treatment

Bracts were forced open to expose fingers a day before the start of flowering and bunches were immersed in four liters of a table salt solution in a transparent polyethylene bag for 24 h (Figure 1). The bunches were then removed from the saline solution at the time of pollination. They were given ample time for the saline solution to drip off and to air dry before performing pollinations with PGM [10]. They were then put back in the saline solution in the evening for the next pollination on the next day, and the procedure was

repeated until all hands were pollinated. Pollinated bunches were then labeled and left to mature in the open. Water was included as a negative control, as summarized in Table 2.

**Figure 1.** A 'Mbwazirume' bunch immersed in a 0.031 M saline solution treatment for seed set increase a day before the start of pollination.

**Table 2.** 'Mbwazirume' bunches pollinated (sample) after salt solution treatment for seed set increase between May and July 2017.

| Salt Concentration (M) | Number of Bunches |
|---|---|
| 0.000 (Control) | 9 |
| 0.031 | 8 |
| 0.063 | 9 |
| 0.125 | 9 |
| 0.250 | 16 |
| 0.500 | 9 |
| 1.000 | 2 |

M, molarity.

### *2.5. Ovule Culture*

Pollinations were performed on two bunches each of 'Calcutta 4', 'Mshale', 'Mlelembo', 'Enzirabahima', and 'Nakitembe', with 'Calcutta 4' pollen using enhanced stigma receptivity as described in Section 2.2 above. The wild banana 'Calcutta 4' was used as the control, since it is highly female-fertile. The fruits were then harvested 48 h post-pollination, with a presumption that fertilization was completed within that time. The fruits were then washed with detergent and surface sterilized in 70% ethanol for 15 min. Under sterile conditions, the fruits were carefully peeled to expose ovules attached to the placenta, which were initiated on standard Murashige and Skoog (MS) media. Because of rapid enzymatic browning, ascorbic acid was added to the culture while some cultures continued no ascorbic acid. Liquid media on a shaker was also experimented in an effort to efficiently control enzymatic browning. Three jars as three replicates were prepared for each media type and treatment (pollinated genotypes), that is, standard MS media, MS media plus abscisic acid, and liquid MS media. The ovules were cultured for up to two months and examined for the presence of embryos.

### 2.6. Seed Extraction

Bunches were harvested when some fruits started to ripen (to yellow). They were then kept in a ripening room, and seeds were hand extracted from the fruit pulp, washed, air-dried, and counted. The number of fingers per bunch was also counted, and seed set was expressed on a per-100 fruit basis as follows:

$$(\text{Total seed in a bunch})/(\text{Total number of fruits of that bunch}) \times 100 \quad (1)$$

This was performed for all of the pollination techniques to standardize seed set, since different bunches had different numbers of fruit fingers. Seed set standardization was not necessary for the ovule culture technique.

### 2.7. Data Analysis

The early and evening pollination techniques (new techniques) were compared with the control pollination technique in a two-sample one-sided *t*-test. The new pollination techniques were considered to be better than the control. For the PGR treatments, a paired *t*-test was performed for the different PGRs and their varying application rates. The PGR-treated bunches were also considered to have yielded more seed than the control. Seed set per 100 fruits of bunches treated with PGRs were averaged for each landrace and each concentration. The corresponding bunches for the control pollination were in the range of pollinations plus or minus 15 days. The plus or minus 15 days range was considered because weather plays a critical role in seed set in bananas. Weather effects are significant from 75 days before pollination until about 10 days before harvest [9]. Data were analyzed using Genstat 19$^{th}$ edition developed by VSN International (VSNi).

## 3. Results

### 3.1. Early and Evening Pollinations

The early and evening pollination techniques did not yield significantly more seed compared to the control pollination technique (Table 3). The mean seed set per 100 fruits per bunch for the control was numerically greater than the new techniques. The evening pollination was the least effective among the three pollination techniques.

**Table 3.** Comparison of seed set per 100 fruits per bunch of early pollination and evening pollination techniques on landrace 'Enzirabahima'.

| Pollination Technique | Bunches Pollinated | Seed/Bunch | t-Probability | Pollination Period |
|---|---|---|---|---|
| Early pollination | 47 | 0.48 | 0.735 | May 2016–April 2017 |
| Control | 37 | 0.66 | | |
| Evening pollination | 34 | 0.27 | 0.884 | June 2016–April 2017 |
| Control | 32 | 0.62 | | |

### 3.2. Hormonal Treatment

All hormonal treatments made on sterile 'Mbwazirume', 'Mlelembo', and 'Nakitembe' did not yield any seed for any of the pollinated bunches (Table 4). However, there was some seed set after hormonal treatment on 'Mshale', 'Nshonowa', and 'Enzirabahima', especially with salicylic acid or TIBA. The t-probabilities for salicylic acid and TIBA were much lower compared to those of other PGRs.

**Table 4.** Plant growth regulator treatment soon after pollination and resulting seed set per 100 fruits compared to the control.

| PGR Name | Rate (ppm) | PGR Treated Bunches | | | | | Control (+/− 15 Days) | | | | t-prob. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Landrace Name | No. Poll. | No. W/Seed | Max. Seed | Av. Seed | No. Poll | No. W/Seed | Max. Seed | Av. Seed | |
| B-Nine | 5000 | 'Enzirabahima' | 5 | 0 | 0.0 | 0.0 | 6 | 1 | 1.4 | 0.2 | |
| | 5000 | 'Mlelembo' | 3 | 0 | 0.0 | 0.0 | 19 | 0 | 0.0 | 0.0 | |
| | 5000 | 'Mshale' | 1 | 1 | 51.9 | 51.9 | 7 | 5 | 30.6 | 12.0 | |
| | 5000 | 'Nakitembe' | 3 | 0 | 0.0 | 0.0 | 27 | 0 | 0.0 | 0.0 | |
| | 5000 | 'Nshonowa' | 7 | 4 | 12.5 | 3.6 | 15 | 13 | 89.7 | 18.4 | |
| Average | | | 3.8 | 1.0 | 12.9 | 11.1 | 14.8 | 3.8 | 24.3 | 6.1 | 0.308 |
| ABA | 500 | 'Enzirabahima' | 5 | 1 | 12.5 | 2.5 | 17 | 4 | 6.5 | 2.2 | |
| | 500 | 'Mlelembo' | 4 | 0 | 0.0 | 0.0 | 18 | 0 | 0.0 | 0.0 | |
| | 500 | 'Mshale' | 2 | 1 | 3.7 | 1.9 | 8 | 6 | 30.6 | 11.5 | |
| | 500 | 'Nakitembe' | 5 | 0 | 0.0 | 0.0 | 14 | 0 | 0.0 | 0.0 | |
| | 500 | 'Nshonowa' | 2 | 0 | 0.0 | 0.0 | 14 | 11 | 26.0 | 9.9 | |
| Average | | | 3.6 | 0.4 | 3.24 | 0.88 | 14.2 | 4.2 | 12.62 | 4.72 | 0.907 |
| 6BAP | 1000 | 'Mshale' | 1 | 0 | 0.0 | 0.0 | 6 | 3 | 26.8 | 20.2 | |
| | 2000 | 'Mshale' | 1 | 0 | 0.0 | 0.0 | 5 | 3 | 26.8 | 7.8 | |
| Average | | | 1.0 | 0.0 | 0.0 | 0.0 | 5.5 | 3.0 | 26.8 | 14.0 | 0.867 |
| Thiourea | 1000 | 'Mlelembo' | 2 | 0 | 0.0 | 0.0 | 8 | 0 | 0.0 | 0.0 | |
| | 7612 | 'Mbwazirume' | 3 | 0 | 0.0 | 0.0 | 1 | 0 | 0.0 | 0.0 | |
| | 15224 | 'Nakitembe' | 1 | 0 | 0.0 | 0.0 | 11 | 0 | 0.0 | 0.0 | |
| | 38060 | 'Nakitembe' | 1 | 0 | 0.0 | 0.0 | 11 | 0 | 0.0 | 0.0 | |
| Average | | | 1.8 | 0.0 | 0.0 | 0.0 | 7.8 | 0.0 | 0.0 | 0.0 | - |
| TIBA | 500 | 'Enzirabahima' | 1 | 1 | 9.3 | 9.3 | 18 | 10 | 15.2 | 3.4 | |
| | 500 | 'Mbwazirume' | 2 | 0 | 0.0 | 0.0 | 3 | 0 | 0.0 | 0.0 | |
| | 500 | 'Mshale' | 1 | 1 | 13.6 | 13.6 | 16 | 12 | 24.6 | 6.4 | |
| | 500 | 'Nakitembe' | 2 | 0 | 0.0 | 0.0 | 24 | 0 | 0.0 | 0.0 | |
| | 500 | 'Nshonowa' | 1 | 0 | 0.0 | 0.0 | 6 | 3 | 7.1 | 1.5 | |
| | 1000 | 'Nakitembe' | 1 | 0 | 0.0 | 0.0 | 17 | 0 | 0.0 | 0.0 | |
| | 1000 | 'Mlelembo' | 1 | 0 | 0.0 | 0.0 | 6 | 0 | 0.0 | 0.0 | |
| Average | | | 1.3 | 0.3 | 3.3 | 3.3 | 12.9 | 3.6 | 6.7 | 1.6 | 0.123 |
| SA spray | 100 | 'Enzirabahima' | 2 | 1 | 0.9 | 0.5 | 6 | 3 | 7.6 | 1.8 | |
| | 200 | 'Enzirabahima' | 1 | 1 | 7.1 | 7.1 | 14 | 9 | 10.7 | 2.3 | |
| SA injection | 200 | 'Mlelembo' | 1 | 0 | 0.0 | 0.0 | 6 | 0 | 0.0 | 0.0 | |
| | 100 | 'Mshale' | 1 | 1 | 75.0 | 75.0 | 2 | 2 | 38.2 | 36.0 | |
| | 100 | 'Nshonowa' | 1 | 1 | 5.7 | 5.7 | 8 | 4 | 29.2 | 5.5 | |
| Average | | | 1.2 | 0.8 | 17.7 | 17.7 | 7.2 | 3.6 | 17.1 | 9.1 | 0.164 |

PGR, plant growth regulator; PGR treated bunches rate (ppm), application/spray rate measured in parts per million; No. poll., number of bunches pollinated (sample); No. w/seed, number of bunches with seed; Max. seed, maximum seed per bunch per 100 fruits; Av. seed, average seed per 100 fruits per bunch; t-prob., paired *t*-test t-probability for comparison of seed set after plant growth regulator treatment and the control (no treatment); ABA, abscisic acid; 6BAP, 6-benzylamino purine; TIBA, triiodo benzoic acid; SA, salicylic acid.

### *3.3. Saline Solution Treatment*

All bunches harvested from the saline solution treatment, before pollination, did not yield any seed. The high concentration of 1 M had phytotoxic effects on young bunches; thus, it was discontinued after pollinating two bunches. During seed extraction, it was noticed that salt-treated fruits had more large ovules that were presumed to have been fertilized, but withered compared to the control.

### *3.4. Ovule Culture*

The wild banana 'Calcutta 4' grew on MS media for up to 42 days, though some of the jars showed enzymatic browning (Figure 2A,B). Mchare landraces, including 'Mshale', 'Mlelembo', and 'Nshonowa' also had some cultures showing growth; however, the ovules remained mostly undersized and withered (Figure 2C). 'Mlelembo' showed the best response among the Mchare landraces, and it also had fruit pulp development (Figure 2C). On the other hand, Matooke landrace ovules withered shortly after initiation, leaving behind plenty of phenolic compounds in the media (Figure 2D,E). An attempt to disperse phenolics into the media as soon as they were released by using liquid medial on a shaker did not yield any growth response (Figure 2F). The use of high levels of ascorbic acid did not efficiently control phenolics release. Cultures with and without ABA did not yield proper ovule growth. The ovules of Mchare and 'Calcutta 4' that grew up to 42 days on MS media did not yield any embryos for in vitro germination.

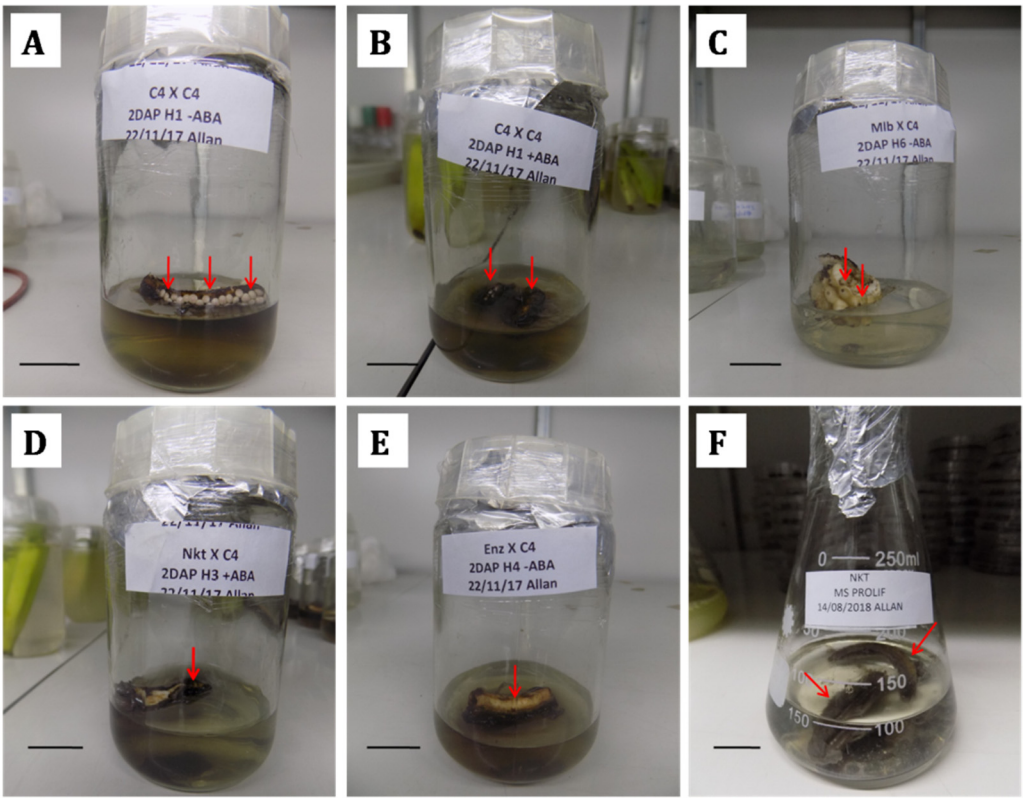


**Figure 2.** Wild banana 'Calcutta 4' and East African Cooking banana ovules in culture initiated two days after pollination (indicated with red arrows). Pictures were taken 20 days after initiation (**A**–**E**), bars = 2 cm. (**A**) Growing 'Calcutta 4' ovules with some browning from the first hand. (**B**) 'Calcutta 4' ovules engulfed in phenolics from the first hand. (**C**) Slow growing 'Mlelembo' ovules from the sixth hand. (**D**) Withered 'Nakitembe' ovules from the third hand. (**E**) Intense browning of 'Enzirabahima' ovules from the fourth hand. (**F**) Failed initiation of 'Nakitembe' ovules 43 days after initiation in liquid MS media.

## 4. Discussion

Most conventional banana improvement programs have screened landraces and selected those with considerably high fertility for genetic hybridization. For example, IITA in Nigeria selected 12 plantain landraces [12], whereas in Uganda, 37 out of 78 EAHBs are considered fertile [23]. Quite often, the most preferred landraces are not included in crossing schemes because they have a very poor seed set or have been rendered "infertile". For example, among the EAHBs, 'Nakitembe' and 'Mbwazirume' are some of the most preferred landraces, but they do not set seed [23,25]. The selected landraces have,

therefore, been "overused" in sexual hybridization, thus exhausting genetic variability in these particular landraces. The only changes made are the male parents used; yet, it is essential to change female parents as well. Consequently, the available gene pool for given banana types in various genetic improvement programs has been under-utilized. There is a need to increase seed set and/or overcome sterility in bananas, but no consistent deliberate measures have been put in place to achieve this goal.

Shepherd (1954) [31] found out that increasing moisture around freshly pollinated bunches increased seed set; however, the numbers were not significant. On the other hand, the use of PGM during pollination significantly increases seed set in bananas [10]. The increase is a small fraction of the potential seed set, as the vast majority of ovules abort after pollination [11]. In the Matooke landrace 'Enzirabahima', PGM has been shown to increase seed set per 100 fruits per bunch from 1.6 to 2.8 seeds [10], but the potential seed set is 36,540 seed per 100 fruits [11]. Additionally, seed set increase with the use of PGM has happened in seed-fertile landraces; thus, there is a need to explore other breeding techniques to break pollination barriers and broaden the parental base for sexual hybridization in improvement programs.

Self-incompatibility (SI) is not yet well understood, but it has been suggested to occur in bananas. The type of SI that occurs in bananas is gametophytic SI, where pollen tubes are arrested in the style [31]. Bananas are usually pollinated with more than enough pollen to fertilize all ovules [8], but the majority of pollen tubes are arrested and do not reach the ovules [31]. This suggests a SI system in bananas. SI in *Musa* spp. is also supported by the observation of pollination of closely related *M. acuminata* Colla and *M. balbisiana* Colla clones, which yielded more seed than self-pollinations [32]. The rationale of early pollination was, therefore, for pollen tubes to reach the ovules before the formation of glycoproteins, which are said to be responsible for SI. Early pollination may not have worked because premature stigmas are said to delay or prevent penetration by pollen tubes [33]. In *Citrus*, a study demonstrated that bud pollination is the most efficient means of overcoming SI, compared to stress treatment and chromosome doubling [34]. The approach of early pollination was not effective for bananas, and thus it may require modifications to become effective.

Banana anthesis starts at about 15:00 h and continues through the night until about 09:00 h of the next day, depending on the genotype, group, and sex of the flowers [29]. Evening pollination was aimed at having ovules fertilized as soon as possible. Ovules are said to disintegrate within 24 h of flower opening [8]; thus, pollination as soon as flowers open is critical. This endeavor did not work, because the male parent that was used, 'Calcutta 4', opens its male flowers between 01:00 and 06:30 h [29]. This implied that pollen viability had reduced at the time of pollination in the evening [8]. However, there are other genotypes whose male flowers open as early as 18:20 h [29] that could be used as pollen sources if they have traits of interest. Additionally, techniques that overcome pollen tube inhibitors have to be used in combination with evening pollination.

Salt treatment is used in brassicas to overcome SI through inactivation of proteins that cause it on the stigmatic surface [35]. In this study, the approach was to dip banana bunches in a saline solution long enough for the salt to be absorbed into the styles. However, this was not enough to overcome sterility in the seed-sterile banana landrace. More presumably fertilized large ovules observed in saline treated bunches suggest that more pollen tubes reached the ovules and resulted in fertilization. Pollen tube arrest in the styles is, therefore, not the major contributor to sterility, as there was no seed set increase in the seed-sterile landraces.

All plant physiological processes are virtually driven by PGRs, and this applies to seeded and seedless phenotypes in bananas. Among the PGRs tried, salicylic acid, which interferes with auxin signaling and the auxin transport inhibitor TIBA, may potentially unlock the female sterility mystery in bananas. PGR-treated bunches that set seed were few; thus, these results cannot be considered very reliable. In a period of plus or minus 15 days, there were bunches that set more seed than those treated with PGRs. This implies that seed

set after treatment with PGRs could have been a coincidence. Additionally, PGRs had no effect on the inherently sterile 'Mlelembo', 'Mbwazirume', and 'Nakitembe' landraces. For PGRs to be effective, the right ones have to be identified and applied at the right time, at an optimum rate, and in the right way.

It was very difficult to control the phenolic compounds released in the experimental ovule cultures; thus, ovule culture is a less viable option for overcoming sterility in bananas. Even with successful initiation of the cultures, embryos did not develop. This could have been as a result of early harvesting of fruits before embryos were big enough for ovule culture. In seedless bunch grape ovule cultures, samples that were picked 60 days after pollination yielded the highest percentage of embryos compared to 40, 20, and 10 days after pollination [22]. Unfortunately, banana ovules abort about 14 days after pollination; thus, they cannot be picked when embryos have had considerable development [11]. Abscisic acid, which has been implicated in seed development, also did not have an effect on ovule development in culture.

## 5. Conclusions

Banana breeding programs have screened edible bananas for female fertility and have selected a set of fertile ones for sexual hybridization. Seed set from these female fertile genotypes is still far below the potential, and they have been used for a long time. Overcoming pollination barriers in edible female sterile banana genotypes is a key step towards expanding the parental base and creating a wider progeny base for selection. The use of PGM in pollinations can increase seed set by more than 50%, but only in female-fertile genotypes. Other pollination techniques were, therefore, explored to overcome pollination barriers. Early pollination may have to undergo modifications to have an effect in bananas, as this method did not show promising results. For evening pollination to work, both male and female parents have to open at about the same time before night fall. Ovule culture cannot be used for overcoming sterility in bananas, but the use of PGRs, especially auxin antagonists, should be given more attention, as results were inconclusive. Saline solution treatment showed promise in increasing ovule fertilization rates, but this has to be used in combination with other pollination techniques to be successful.

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