

## GENETIC DIVERSITY AMONG EAST AFRICAN HIGHLAND BANANAS FOR FEMALE FERTILITY

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### ABSTRACT

There are 84 distinct cultivars of highland bananas (*Musa* spp.) in Uganda, grouped in five clone sets and it is not known which among these are female fertile. The objective of the study reported herein was to identify female fertile highland bananas that can be used in a cross breeding program and to determine the influence of pistil morphological traits on seed set. Seventy eight cultivars were screened for female fertility using pollen from 'Calcutta 4' and thirty-three cultivars representing the major variability of highland bananas were selected for studying pistil morphological traits to establish their relationship with seed yield. The clone sets and cultivars were highly significantly ( $P \leq 0.0001$ ) different in seed set rates but between-clone effects were higher than within-clone effects. Number of seeds was significantly negatively correlated with style length, ovary length and diameter of the style base. Path analysis indicated a high residual, suggesting that the traits analysed had little influence on female fertility. Nevertheless, style length was the most important trait in determining seed set because it had the strongest direct effect. Abnormalities in pistil morphological traits render cultivars female sterile.

*Key Words:* Highland bananas, *Musa* spp., pistil morphological traits, seed set

### RÉSUMÉ

Il y a 84 variétés distinctes de bananes de montagne (*Musa* spp.) en Ouganda, groupées en cinq ensembles de clones et il n'est pas connu laquelle parmi celles-ci sont des femelles fertiles. L'objectif de cette étude ici rapportée était d'identifier les bananes femelles fertiles des montagnes qui peuvent être utilisées dans un programme reproductif de croisement et de déterminer l'influence des traits morphologiques de pistil sur un groupe des semences. Septante huit variétés étaient épurées pour être utilisées comme pollen femelle fertile à partir de 'Calcutta 4' et trente trois variétés représentant la variabilité majeure des bananes des montagnes étaient sélectionnées pour étudier les traits morphologiques du pistil en vue d'établir leurs relations avec le rendement en graines (gamètes). Les groupes des clones et variétés étaient fortement différents de façon significative ( $P = 0,0001$ ) en taux de groupe de graines mais entre les clones les effets étaient forts qu'entre les effets de clones. Le nombre des semences était négativement corrélé de façon significative avec la longueur du style, la longueur de l'ovaire et le diamètre de base de style. L'analyse pathologique a indiqué un fort effet résiduel suggérant que les traits analysés avaient une faible influence sur la fertilité féminine. Néanmoins, la longueur du style était le plus important trait dans la détermination de groupe de semences parce qu'elle avait le plus fort effet direct. Les irrégularités en traits morphologiques de pistil rendent les variétés femelles stériles.

*Mots Clés:* Bananes de montagne, *Musa* spp., traits morphologiques du pistil, groupe de semences

## INTRODUCTION

Banana (*Musa* spp.) belongs to the family *Musaceae* and has its origin in South East Asia (Simmonds, 1962). Most of the cultivated types are believed to have been derived by natural hybridization between the diploid wild species *M. acuminata* Colla and *M. balbisiana* Colla, which contributed the A and B genomes, respectively (Simmonds and Shepherd, 1955; Simmonds, 1966). The edible bananas have 22, 33, or 44 chromosomes (the basic number being  $x = 11$ ). Among the edible *Musa* cultivars, triploids are generally the most numerous, diploids less so and tetraploid forms are very rare. The most important cultivars vary in their genomic constitution and are generally categorized as follows: AAA, with most of the dessert bananas and East African highland bananas; AAB with the plantains and some dessert bananas; ABB with other cooking and juice producing bananas plus a few dessert bananas (Simmonds, 1987). There are also important diploids (AB, AA) which are used as dessert or juice producing bananas.

The East African highland bananas (Shepherd, 1957) constitute 85% of all the bananas grown in Uganda and are the most important food crop in the country (INIBAP, 1999). Unfortunately, banana production per unit land area in the traditional banana growing areas in Uganda has steadily declined since the 1970s (FAO, 1991), with current yields standing at 4.5 to 6.0 t ha<sup>-1</sup> compared to the potential 60 to 90 t ha<sup>-1</sup> (KARI, 1996). This threatens the food security and cash incomes of the people. This decline is mainly attributed to pests and diseases. Among the pests, the banana weevil (*Cosmopolites sordidus*) and a complex of plant parasitic nematodes are the most important (Gold *et al.*, 1993). Weevil infestation, which is widespread in almost all the banana growing areas of Uganda, can cause 50 to 100% yield loss and reduced life span of the plantation (Gold *et al.*, 1993). Although several nematode species occur in banana plantations in Uganda, the most important are *Pratylenchus goodeyi*, *Helicotylenchus multicinctus* and *Radopholus similis* (Kashaija *et al.*, 1994). Yield loss of up to 50% due to nematodes has been reported on the highland bananas (Speijer and Kajumba, 1996).

Among the diseases, black sigatoka, caused by the air borne fungus *Mycosphaerella fijiensis*, is the most important disease of highland bananas (Tushemereirwe and Waller, 1993). Yellow sigatoka (*M. musicola*) and *Cladosporium* speckle (*C. musea*) are other leaf spot diseases but considered of minor importance (Leaky, 1970; Tushemereirwe, 1996). Studies at Kawanda Research Institute, Uganda indicated that this leaf spot complex can reduce yield by 37% (KARI, 1996). Banana streak disease caused by a badnavirus was first reported in Uganda in 1996 (Tushemereirwe *et al.*, 1996) and can cause up to 100% yield loss (KARI, 2002).

Host plant resistance is considered to be the most economically sustainable plant protection strategy (Stover and Simmonds, 1991). However, a constant hindrance in the conventional breeding of edible *Musa* spp. has been the low reproductive fertility. Seed set rate is a characteristic of the cultivar (Simmonds, 1960; Vuylsteke *et al.*, 1993). Structural hybridism, which affects both male and female flowers, is a major cause of reproductive sterility. When two species are crossed, the chromosomes from each parent may not pair regularly at meiosis in the hybrid. Consequently, whole chromosomes or parts of chromosomes are lost and the nuclei of the pollen grains and ovules are genetically incomplete leading to inviable gametes and hence sterility. Apart from the question of structural hybridism, triploids are invariably less fertile than diploids. During gametogenesis in a triploid, the chromosomes are distributed irregularly to the daughter nuclei. Only nuclei containing complete and balanced chromosome sets form viable gametes (Shepherd, 1960). Triploids of the genomic constitution ABB have been reported to be more fertile than those of the AAB or AAA genome (Shepherd, 1960 and Ortiz and Vuylsteke, 1995). Seed production in *Musa* is also under the influence of environmental factors (Shepherd, 1954; Swennen *et al.*, 1991 and Ortiz and Vuylsteke (1995), which may account for differences in fertility among localities. Seed production in cultivars with short styles has been reported to be higher than that in cultivars with long styles (Shepherd, 1954, 1960; PBIP, 1993). In a similar study, a comparison of the distribution

of seeds in the fruits of eight cultivars revealed apical bias of seed set being a general phenomenon in edible bananas suggesting that pollen tubes have problems traversing long distances (Shepherd, 1960).

In Uganda, the current figure for the number of distinct cultivars of highland bananas is 84, which have been classified into five clone sets based on overall similarities of morphological characters as follows (Karamura, 1998):

**Mbidde clone set.** The clones have an astringent and bitter tasting pulp caused by the presence of more tannins than in the cooking clones. Before maturity, pulp colour is white with sticky brown excretions across the pulp, while after maturity, it is cream with sticky brown excretions across the pulp. The clones can have characters of other clone sets.

**Musakala clone set.** Clones have pendulus and lax bunches, with long fruits above 20cm. Fruits have bottle necked apices and pendulus male inflorescence rachis. Fruit pulp is insipid, with no brown sticky excretions across the pulp.

**Nakabululu clone set.** Clones have sub-horizontal to very compact bunches. Fruits are short (below 15cm) with blunt apices. They have sub-horizontal to oblique nude male inflorescence rachis. Fruit pulp is insipid, with no brown sticky excretions across the pulp.

**Nakitembe.** Clones have oblique compact bunches, with fruits of medium length (15 – 20 cm). Fruit apices are intermediate between bottle-necked and blunt. The male inflorescence rachis has persistent bracts and neuter flowers. Fruit pulp is insipid, with no brown sticky excretions across the pulp.

**Nfuuka clone set.** Clones have oblique compact bunches with medium fruits of 15 – 20 cm long. Fruit apices are intermediate between bottle-necked and blunt. They have oblique or sigmoid curved nude male inflorescence rachis. Fruit pulp is insipid, with no brown sticky excretions across the pulp.

It is not known which of these cultivars are female fertile. The objective of this work was therefore to screen the highland bananas for female fertility so as to identify those that can be used in a cross breeding program and to establish the relationship between seed set and pistil morphology.

## MATERIALS AND METHODS

**Plant Materials.** The highland banana cultivars maintained in the field gene bank at Kawanda Agricultural Research Institute, Uganda were used for the study. These included 11, 34, 11, 10 and 12 cultivars from Musakala, Nfuuka, Nakitembe, Nakabululu, and Mbidde clone sets, respectively. The cultivars used in the study differ in bunch characteristics, fruit characteristics and characteristics of the male inflorescence (Karamura, 1998). All the cultivars are publicly available for both research and production purposes. Nakabululu and Nfuuka are the least distinct clone sets. Nfuuka and Nakitembe are the next least distinct, while Musakala and Beer are very distinct from each other and from the rest of the clone sets. This classification is based on morphological traits and not on molecular and biochemical characteristics. All the available plants were used for the study totaling to 76, 696, 80, 132 and 116 plants for Musakala, Nfuuka, Nakitembe, Nakabululu, and Mbidde clone sets, respectively. The number of plants/bunches pollinated per cultivar varied from 1 to 69. Several factors were responsible for the variation, e.g.,

- The experiment was conducted in a banana germplasm collection, to which new entries were continuously being added. Thus, more plants were pollinated over time for the old entries than for the new entries.
- The different accessions had different sucker producing capacities. Thus, more plants were available for highly prolific accessions than for less prolific ones.
- The different accessions had different maturity periods. Thus, within a given period of time,

more plants were available for the early than for the late maturing varieties.

- Resistance to pests, diseases and drought played a role in varying the number of plants available for pollination over time.
- The same germplasm collection was also used by other researchers for different experiments. This was inevitable because banana research is relatively expensive (each plant requires 6 to 9 m<sup>2</sup>) and one way of reducing research costs is to use few fields for several experiments.

**Screening for female fertility.** Seed set in *Musa* is influenced by cultivar (genotype) among other factors. Thus, some cultivars are sterile, others are low and medium seed producers, while some are high seed producers (Simmonds, 1960). The seed producing ability of the highland banana accessions maintained at Kawanda (Uganda) is not known. In order to identify female fertile highland bananas that can be used in a crossbreeding program, pollen from *M. acuminata* subsp. *burmanicoides* 'Calcutta 4', a highly male fertile accession (De Langhe and Devreux, 1960), was used to pollinate female inflorescences of the 78 accessions available at Kawanda (32°32' E, 0°25' N, 1177 meters above sea level). The climate at Kawanda is moist sub-humid with a mean annual rainfall of 1250 mm, bimodally distributed with peaks from March – May and September – November. Daily temperatures at Kawanda average 15.3°C minimum and 27.3°C maximum, while relative humidity is 76.3%. The soil at Kawanda is sandy loam (or deep ferrallitic clay type) with pH of 5.5-6.0.

The study was conducted from January 1995 to March 1997, and pollination was conducted as described by Ortiz and Vuylsteke (1995). Pollen from 'Calcutta 4' was always collected around 0630 hours from the male buds previously covered with bags made of cotton material at anthesis, to prevent pollen contamination with other sources (Mutsaers, 1993). Likewise, emerging inflorescences of the highland bananas were bagged with transparent plastic bags, to avoid potential natural crossing with alien pollen. Hand

pollinations were performed daily between 0630 and 0730 hours when flowers were more receptive (Shepherd, 1954; 1960) in freshly exposed female flowers of the highland bananas by rubbing a cluster of anthers (with pollen) from 'Calcutta 4' on to the stigmas of the highland bananas. Pollinations were carried out for all the stigmas in the individual hands as the bracts opened to expose the stigmas. Pollinated bunches were labeled with tags indicating cross number, parents and initial pollination date. The plastic bags were removed from the inflorescence a day after the last hand was pollinated. The available 11, 34, 11, 10 and 12 cultivars were pollinated for Musakala, Nfuuka, Nakitembe, Nakabululu and Mbidde clone sets, respectively. A total of 76, 696, 80, 132 and 116 bunches were pollinated for the respective clone sets. At full maturity (approximately 90 days following pollination), bunches were harvested, placed in a ripening room and allowed to naturally ripen for about seven days. Seeds were then manually extracted for each nodal cluster (hand), washed, air-dried and counted.

A record was made of total number of bunches pollinated, number of bunches with seeds and number of seeds produced. Pollination success was computed as the percentage of bunches with seeds over total number of bunches pollinated.

Data were analysed for 48 cultivars (those with at least seven pollinated bunches) using SAS software (SAS, 1998) after a cube root transformation to linearise the data (Emerson and Stoto, 1983). Mixed Model analysis was performed taking clone sets and cultivars as fixed effects and years and months as random effects (Littell *et al.*, 1996), based on the following equation:

$$Y_{ijkl} = \mu + s_i + c_{j(i)} + y_k + m_l + (sy)_{ik} + (cy)_{jk} + (sm)_{il} + e_{ijkl}$$

where:  $Y_{ijkl}$  is the observed seed yield for the  $j^{\text{th}}$  cultivar within the  $i^{\text{th}}$  clone set pollinated in the  $l^{\text{th}}$  month of the  $k^{\text{th}}$  year.

$\mu$  is the general mean.

$s_i$  is the  $i^{\text{th}}$  clone set effect ( $i = 1, 2, \dots, 5$ ).

$c_{j(i)}$  is the  $j^{\text{th}}$  cultivar within the  $i^{\text{th}}$  clone set effect ( $j = 1, 2, \dots, 48$ ).

$y_k$  is the  $k^{\text{th}}$  year effect ( $k = 1, 2, 3$ )

$m_l$  is the  $l^{\text{th}}$  month effect ( $l = 1, 2, \dots, 12$ )

$(sy)_{ik}$  is the interaction between  $i^{\text{th}}$  clone set and  $k^{\text{th}}$  year

$(cy)_{jk}$  is the interaction between  $j^{\text{th}}$  cultivar and  $k^{\text{th}}$  year

$(sm)_{il}$  is the interaction between the  $i^{\text{th}}$  clone set and  $l^{\text{th}}$  month

$e_{ijkl}$  is the residual effect (error) for the  $j^{\text{th}}$  cultivar within the  $i^{\text{th}}$  clone set in the  $l^{\text{th}}$  month of the  $k^{\text{th}}$  year.

**Pistil morphological traits.** The morphology of the pistil may affect the seed producing ability and may account for differences in seed set rates among the genotypes/clone sets of highland bananas. To verify this assertion in highland bananas, 33 cultivars representing the major variability of highland bananas in Uganda were used for detailed studies on pistil morphological studies. The trial was established at Namulonge (32°35' E, 0°32' N, and 1150 meters above sea level). Namulonge has a mean annual rainfall of 1000-1200 mm, relative humidity of 80%, and annual temperatures of 16-30°C. The soils are sandy-clay loam, with a pH of 5.2-6.0. A total of 6, 15, 3 and 9 cultivars were selected from Musakala, Nfuuka, Nakitembe and Nakabululu clone sets, respectively. Two ovaries were randomly selected from each of the first, second and third hands for detailed studies of pistil morphological traits.

Data were recorded on the diameter of the stigma at its widest cross section (mm), length of the style (cm), length of the ovary (cm), diameter of the style base at its widest cross section (mm) and number of ovules in the ovary. The accessions were then pollinated using pollen from 'Calcutta 4' as described above. Data were analysed using SAS software (SAS, 1998). Analysis of variance was performed for pistil morphological traits and means were separated using Tukey's Studentized Range Test (Steel *et al.*, 1997). Correlation

coefficients among pistil morphological traits and average seed set per cultivar were assessed. Path coefficients for direct and indirect effects (Dewey and Lu, 1959) were calculated using traits that were significantly correlated with seed yield and/or with each other. The analysis was carried out taking seed yield as the effect determined by means of pistil morphological traits (the cause), following the method described by Ortiz and Langic (1997).

## RESULTS AND DISCUSSION

**Covariance parameter estimates for random effects and their interactions.** The covariance parameter estimate for months was significant ( $P \leq 0.01$ ) suggesting that pollinations carried out in the different months yielded different amounts of seed. However, the effects of years and the interactions of years/months with clones and clone sets were non-significant. Further analysis indicated that seed set was high with pollinations made in February and March, a period that was characterised by high temperatures, high solar radiation and low relative humidity (Ssebuliba, 2003). Ortiz and Vuylsteke (1995) obtained similar results in two plantain cultivars under West African environment conditions.

**Seed set in the different clone sets of highland bananas.** Clone sets were highly significantly ( $P \leq 0.0001$ ) different in seed set (Table 1). Nakabululu and Nfuuka clone sets had the highest pollination success of 49.2% and 45.1%, respectively. The two clone sets also had high average seed set rates of 5.06 and 5.52 seeds per bunch, respectively, as opposed to only 0.17, 0.01 and 0.49 for Musakala, Nakitembe and Mbidde, respectively. These results suggest that Nfuuka and Nakabululu clone sets were highly female fertile while Musakala, Nakitembe and Mbidde clone sets had low or negligible female fertility. Female fertility studies in plantains also revealed that the different types or groups of plantains (Tezenas du Montcel *et al.*, 1983) also differ in fertility rates (Vuylsteke *et al.*, 1997).

**Seed set rates in individual cultivars of highland bananas.** There were significant ( $P \leq 0.0001$ ) differences among the cultivars in seed set per

bunch (Table 2). Out of the 78 cultivars pollinated, 37 produced seed. Seed set in individual cultivars ranged from 0 to about 25 seeds per bunch. Within Nfuuka clone set, the best cultivars in average seed set were 'Entukura' 'Enzirabahima', 'Kabucuragye' and 'Nante' with 25.1, 18.1, 12.8 and 12 seeds per bunch, respectively. These were followed by 'Tereza' (9.2), 'Enyeru' (7.8), 'Nakitengwa' (7.2) and 'Namafura' (5.0). Within Nakabululu clone set, 'Kazirakwe', 'Nakasabira' and 'Nakayonga' produced the highest number of seeds per bunch, i.e., 11.0, 8.1 and 7.4, respectively.

The relative contribution of between clone effects and within clone effects was highly significant (Table 3). However, the contribution of between clone effects was higher than the within clone effects suggesting that selection of fertile clones should target specific clone sets. Hence fertile clones should be selected from Nfuuka and Nakabululu clone sets.

**Pistil morphological traits.** There were significant differences among clone sets for the different pistil morphological traits (Table 4). Style length, ovary length, number of ovules and diameter of the style base were significantly negatively phenotypically correlated ( $P \leq 0.001$ , 0.05, 0.05 and 0.05, respectively) with seed yield (Table 5). The correlation coefficients among the pistil morphological traits were all positive and significant, with the exception of number of ovules

with diameter of the style base and length of the style, which were non-significant.

The direct effects of style length, ovary length, number of ovules and diameter of the style base on seed set were negative (Table 6). Style length had the strongest direct effect of -0.359, accounting for about 70% of the observed correlation (-0.515) between seed yield and style length. The direct effect of ovary length on seed yield accounted for only 36% of the observed correlation between ovary length and seed yield (-0.487) while that of the style base was the weakest.

The indirect effects of style length, ovary length, number of ovules and diameter of the style base on seed yield were all negative but generally weak (Table 6). The strongest indirect effect was that of ovary length through style length (-0.151, close in magnitude to the direct effect of ovary length). This indirect effect was due to the significant positive correlation observed between style length and ovary length. The indirect effect of diameter of style base through style length (-0.140) was stronger than the direct effect of diameter of the style base (-0.124). The indirect effect of number of ovules on seed yield through ovary length was stronger (-0.133) than the direct effect (-0.103), due to the highly significant positive correlation between ovary length and number of ovules per ovary ( $P \leq 0.001$ ).

The results above suggest that among all the traits studied, style length was the most important

TABLE 1. Seed set by clone set in highland bananas, pollinated with pollen from 'Calcutta 4' from January 1995 to December 1997

Clone Set	No. of cultivars	No. of seed-fertile cultivars	No. of bunches pollinated	No. of bunches with seed	Pollination success (%)	Total seed set	Seed set <sup>x</sup> per bunch	Seed set <sup>y</sup> per bunch	LS Means <sup>z</sup>
Musakala	11	2	76	3	3.9	13	0.17	0.22	0.81 <sup>b</sup>
Nfuuka	34	26	696	314	45.1	3843	5.52	5.83	1.30 <sup>a</sup>
Nakitembe	11	1	80	1	1.3	1	0.01	0.00	0.75 <sup>b</sup>
Nakabululu	10	4	132	65	49.2	668	5.06	5.26	1.23 <sup>a</sup>
Mbidde	12	4	116	10	8.6	57	0.49	0.51	0.85 <sup>b</sup>
Total	78	37	1100	393	-	4582	-	-	-
Mean	-	-	-	-	35.7	-	4.17	4.52	-

Means followed by the same letter are not significantly different according to the pair-wise comparison of LS Means

<sup>x</sup> means based on total number of cultivars pollinated in each clone set

<sup>y</sup> means based on cultivars with at least seven bunches pollinated

<sup>z</sup> means obtained by analysing for clones with at least seven bunches pollinated. Analysis was done after a cube root transformation

TABLE 2. Seed set in East African highland banana landraces pollinated with pollen from 'Calcutta 4'

Clone	No. bunches pollinated	No. bunches with seed	Pollination success	Total seed set	Seed set per bunch	LS Means <sup>Z</sup>	Max. No. seeds
<b>Musakala</b>							
Lumenyamagaali	9	0	0.0	0	0.0	0.83 n-r	0
Murure	6	0	0.0	0	0.0		0
Mugishu agenda	9	0	0.0	0	0.0	0.82 n-r	0
Mukazi alanda	13	2	15.4	2	0.2	0.78 r	1
Musakala	2	0	0.0	0	0.0		0
Mutuliti	3	0	0.0	0	0.0		0
Muvubo	12	0	0.0	0	0.0	0.71 r	0
Nakibizzi	3	0	0.0	0	0.0		0
Nalugolima	10	1	10.0	11	1.1	0.97 n-r	11
Namunwe	7	0	0.0	0	0.0	0.74 r	0
Wansimirahi	2	0	0.0	0	0.0		0
<b>Nfuuka</b>							
Atwalira	16	0	0.0	0	0.0	0.63 r	0
Bitambi	40	8	20.0	56	1.4	0.99 n-r	32
Enjeriandet	29	10	34.5	55	1.9	1.08 k-r	22
Entukura	34	31	91.2	852	25.1	2.36 a	227
Enyeru	25	14	56.0	196	7.8	1.60 d-h	56
Enzinga	6	0	0.0	0	0.0		0
Enzirabahima	43	35	81.4	777	18.1	2.22 ab	100
Enzirabushera	6	1	16.7	0	0.2		1
Kabucuragye	32	25	78.1	411	12.8	1.94 bc	65
Karinga	3	1	33.3	1	0.3		1
Kibalawo	4	0	0.0	0	0.0		0
Kulwoni	1	0	0.0	0	0.0		0
Lusumba	9	4	44.4	13	1.4	1.07 k-r	9
Nabusa	4	0	0.0	0	0.0		0
Nakabinyi	69	26	37.7	110	1.6	1.12 k-q	18
Nakawere	42	21	50.0	81	1.9	1.22 i-p	11
Nakhaki	8	1	12.5	8	1.0	0.96 n-r	8
Nakinyika	17	5	29.4	19	1.1	1.03 k-r	10
Nakitengwa	13	10	76.9	93	7.2	1.69 c-g	35
Namafura	2	1	50.0	10	5.0		10
Namamuka	2	1	50.0	1	0.5		1
Namande	26	13	50.0	90	3.5	1.31 g-l	18
Namayovu	18	2	11.1	9	0.5	0.93 n-r	8
Nambogo	3	3	100.0	7	2.3		3
Namwezi	42	19	45.2	144	3.4	1.30 g-m	24
Nante	21	9	42.9	252	12	1.47 f-j	129
Nassaba	4	0	0.0	0	0.0		0
Ndibwabalangira	38	8	21.1	34	0.9	0.95 n-r	8
Nfuuka	44	20	45.5	126	2.9	1.22 i-o	30
Nyabungere	5	1	20.0	9	1.8		9
Nyamanshari	2	0	0.0	0	0.0		0
Siira	10	0	0.0	0	0.0	0.75 r	0
Tereza	42	25	59.5	388	9.2	1.49 f-i	203
Tuulatwogere	36	20	55.6	101	2.8	1.33 g-k	18
<b>Nakitembe</b>							
Bikowekowe	4	0	0.0	0	0.0		0
Enyarutere	2	0	0.0	0	0.0		0
Lisindalo	2	0	0.0	0	0.0		0

TABLE 2. Contd.

Clone	No. bunches pollinated	No. bunches with seed	Pollination success	Total seed set	Seed set per bunch	LS Means <sup>Z</sup>	Max. No. seeds
Mbwazirume	7	0	0.0	0	0.0	0.77 r	0
Nakibule	5	0	0.0	0	0.0		0
Nakitembe	41	0	0.0	0	0.0	0.79 r	0
Namaliga	3	0	0.0	0	0.0		0
Namulondo	8	0	0.0	0	0.0	0.70 r	0
Nandigobe	3	0	0.0	0	0.0		0
Nasaala	3	0	0.0	0	0.0		0
Ntinti	2	1	50.0	1	0.5		1
<b>Nakabululu</b>							
Bukumu	12	0	0.0	0	0.0	0.77 r	0
Kafunzi	1	0	0.0	0	0.0		0
Kazirakwe	26	19	73.1	287	11.0	1.88 cd	52
Kibuzi	9	0	0.0	0	0.0	0.74 r	0
Mukubakonde	10	0	0.0	0	0.0	0.75 r	0
Nakabululu	4	0	0.0	0	0.0		0
Nakasabira	18	15	83.3	145	8.1	1.80 c-f	33
Nakayonga	30	28	93.3	221	7.4	1.83 c-e	28
Nakyatengu	15	0	0.0	0	0.0	0.81 qr	0
Salalugazi	7	0	0.0	0	0.0	0.96 n-r	0
<b>Mbidde</b>							
Bagandeseza	12	0	0.0	0	0.0	0.77 r	0
Endirira	7	0	0.0	0	0.0	0.73 r	0
Engambani	3	0	0.0	0	0.0		0
Enkara	14	6	42.9	48	3.4	1.29 g-n	15
Enshenyuka	1	0	0.0	0	0.0		0
Entundu	19	2	10.5	4	0.2	0.87 n-r	3
Kabula	1	0	0.0	0	0.0		0
Katalibwambuzi	14	0	0.0	0	0.0	0.81 qr	0
Mende	10	0	0.0	0	0.0	0.7 r	0
Mudwale	10	0	0.0	0	0.0	0.80 qr	0
Nalukira	18	1	5.6	1	0.1	0.76 r	1
Namadhi	7	1	14.3	4	0.6	0.92 n-r	4

<sup>Z</sup> means followed by the same letter are not significantly different from each other according to the pair-wise comparison of LS Means

TABLE 3. Relative contribution of between clone and within clone effects to seed variation in highland bananas

Source of variation	Degrees of freedom	Sum of squares	Mean square
Clone sets (between groups)	4	17.38	4.345 <sup>***</sup>
Cultivars/clones (within groups)	43	7.90	0.184 <sup>***</sup>
Total	47	25.28	

<sup>\*\*\*</sup>: Significant at 0.1% probability level



in determining seed set. Cultivars with long styles stand less chance of setting seed, which agrees with reports that pollen tubes that have to travel short distances might have more chances of fertilizing ovules (IITA, 1993). Ortiz and Vuylsteke (1995) obtained an average of 18.2 seeds per bunch in a plantain cultivar 'Bobby Tannap', which had a short style of  $29 \pm 2$  mm. The same researchers also obtained only 3.9 seeds in another plantain cultivar 'Obino I'Ewai' with a long style of  $36 \pm 2$  mm. Apical bias of seeds was found to be a general phenomenon in fruits of edible bananas (Shepherd 1954; 1960), which is also in line with the observation that long distance travel is a problem for pollen tubes and hence a hindrance to seed set in edible bananas.

The short styles observed for Nfuuka and Nakabululu clone sets could partially explain the high seed set rates obtained for the two clone sets. Although the two clone sets on average had pistil morphological traits that favoured high seed set rates, individual cultivars showed differences (Table 7). 'Mukubakonde', a completely female sterile cultivar in Nakabululu clone set, had an

abnormal pistil (Plate 1). The cultivar had a long style of 3.8 cm, the longest among all the cultivars studied. In addition, the style had three pronounced longitudinal grooves, giving an impression of three fused styles, with a vestigial stigma of 3.21 mm diameter as opposed to the general mean of 7.53 mm. It also had very few ovules, about five per ovary, as opposed to 303, the general mean for the cultivars studied. In addition to being few, the ovules were often abnormal (flat not spherical). 'Bukumu', another completely female sterile cultivar from Nakabululu clone set also had a very long style of 3.55 cm, second only to 'Mukubakonde'. It also had a very large style base (7.92 mm diameter) second largest among the cultivars. This wide base was more often than not filled with a yellow substance that at times would protrude to the outside (Plate 2). A similar abnormality was observed in a plantain cultivar 'Agbagba' (De Langhe, 1964) that was found to be almost completely sterile (Vuylsteke *et al.*, 1993). It is suspected that the yellow substance interferes with pollen tube growth. However, the mechanism through which this is done is not

TABLE 4. Pistil morphological traits of the clone sets of East African highland bananas

Clone set	Diameter of the stigma (mm)	Style length (cm)	Ovary length (cm)	No. of ovules	Diameter of the base (mm)
Musakala	8.86 <sup>a</sup>	2.94 <sup>a</sup>	15.14 <sup>a</sup>	345.8 <sup>a</sup>	7.63 <sup>a</sup>
Nfuuka	7.24 <sup>c</sup>	2.83 <sup>b</sup>	10.86 <sup>c</sup>	301.1 <sup>b</sup>	5.67 <sup>c</sup>
Nakitembe	7.67 <sup>b</sup>	2.98 <sup>a</sup>	11.52 <sup>b</sup>	310.4 <sup>b</sup>	5.98 <sup>b</sup>
Nakabululu	6.97 <sup>c</sup>	2.48 <sup>c</sup>	9.19 <sup>d</sup>	264.4 <sup>c</sup>	5.99 <sup>b</sup>
Mean	7.53	2.80	22.39	303.1	6.12
C.V. (%)	8.0	3.7	8.3	8.9	8.4

Means followed by the same letter within a column are not significantly different at the  $P < 0.05$  probability level according to Tukey's Studentized Range Test

TABLE 5. Phenotypic correlation coefficients among pistil morphological traits and seed yield in selected cultivars of highland bananas

Trait	Style length	Ovary length	No. of length	Diameter of style base
Ovary length	0.420*			
No. of ovules	0.329	0.750***		
Diameter of style base	0.389*	0.664***	0.284	
No. of seeds	-0.515	-0.487*	-0.389*	-0.410*

\*, \*\*, \*\*\*: Significant at 5%, 1% and 0.1% probability levels, respectively

TABLE 6. Path coefficients for direct and indirect effects of pistil morphological traits on seed yield

Pathway	Coefficient
<b>Style length</b>	
Direct effect	-0.359
Indirect effect via ovary length	-0.074
Indirect effect via number of ovules	-0.034
Indirect effect via diameter of style base	-0.048
Total correlation	-0.515
<b>Ovary length</b>	
Direct effect	-0.177
Indirect effect via style length	-0.151
Indirect effect via number of ovules	-0.078
Indirect effect via diameter of style base	-0.082
Total correlation	-0.487
<b>Number of ovules</b>	
Direct effect	-0.103
Indirect effect via style length	-0.118
Indirect effect via ovary length	-0.133
Indirect effect via diameter of style base	-0.035
Total correlation	-0.389
<b>Diameter of style base</b>	
Direct effect	-0.124
Indirect effect via style length	-0.140
Indirect effect via ovary length	-0.117
Indirect effect via number of ovules	-0.029
Total correlation	-0.410

clearly understood. The abnormalities noted in the pistils of 'Bukumu' and 'Mukubakonde', as opposed to the pistils of female fertile highland banana cultivars (Plate 3), may account for the observed female sterility of the two cultivars that paradoxically belong to the highly fertile 'Nakabululu' clone set.

The results of the present study indicated that cultivars with short styles generally had higher chances of setting seed than those with long styles. A look at individual cultivars reveals that this was true in many cases but not always. For example, the style length of 'Kazirakwe' was shorter than that of 'Enzirabahima' but the later

produced more seeds. In addition to this, the path analysis for the direct and indirect effects of pistil morphological traits on seed yield indicated a high residual. These observations suggest that the traits analyzed had little influence on female fertility, implying that there were other factors playing a bigger role in determining female fertility in these cultivars. These factors could be related to stigma receptivity, ovule receptivity, fertilization process, and embryo survival (Sheperd, 1960).

This study provides an insight into the fertility of highland bananas and it is the basis for future

TABLE 7. Pistil morphological traits (mean  $\pm$  SE) of 33 selected cultivars

Cultivar	N	Diameter of the stigma (mm)	Style length (cm)	Ovary length (cm)	No. of ovules	Diameter of the style base (mm)
<b>Musakala clone set</b>						
Mukazi alanda	60	7.85 $\pm$ 0.17	2.77 $\pm$ 0.05	12.01 $\pm$ 0.35	354.8 $\pm$ 12.9	6.39 $\pm$ 0.16
Murure	30	9.00 $\pm$ 0.19	3.16 $\pm$ 0.05	16.14 $\pm$ 0.54	390.3 $\pm$ 13.2	7.70 $\pm$ 0.29
Musakala	66	8.98 $\pm$ 0.21	3.17 $\pm$ 0.02	17.03 $\pm$ 0.41	344.7 $\pm$ 12.6	7.88 $\pm$ 0.18
Muvubo	36	9.25 $\pm$ 0.38	3.18 $\pm$ 0.03	15.46 $\pm$ 0.50	350.4 $\pm$ 12.7	7.72 $\pm$ 0.08
Nakibizzi	18	8.72 $\pm$ 0.80	3.02 $\pm$ 0.01	14.81 $\pm$ 1.37	328.9 $\pm$ 35.6	7.67 $\pm$ 0.82
Namunwe	42	9.76 $\pm$ 0.21	2.40 $\pm$ 0.04	15.78 $\pm$ 0.54	307.7 $\pm$ 7.4	8.89 $\pm$ 0.28
<b>Nfuuka clone set</b>						
Bitambi	72	7.06 $\pm$ 0.13	2.97 $\pm$ 0.02	10.90 $\pm$ 0.12	280.7 $\pm$ 5.7	5.51 $\pm$ 0.15
Enzirabahima	36	7.19 $\pm$ 0.13	2.74 $\pm$ 0.02	11.09 $\pm$ 0.19	317.5 $\pm$ 5.1	5.83 $\pm$ 0.17
Kabucuragye	24	7.54 $\pm$ 0.24	2.88 $\pm$ 0.07	12.37 $\pm$ 0.41	324.2 $\pm$ 18.2	6.15 $\pm$ 0.34
Kibalawo	42	6.50 $\pm$ 0.34	2.62 $\pm$ 0.05	9.10 $\pm$ 0.38	269.9 $\pm$ 6.4	4.95 $\pm$ 0.17
Kulwoni	42	6.67 $\pm$ 0.19	2.56 $\pm$ 0.02	10.28 $\pm$ 0.11	317.7 $\pm$ 9.2	5.33 $\pm$ 0.12
Nabusa	48	7.16 $\pm$ 0.24	2.95 $\pm$ 0.03	10.79 $\pm$ 0.34	293.3 $\pm$ 10.3	5.58 $\pm$ 0.18
Nakawere	42	7.36 $\pm$ 0.12	2.85 $\pm$ 0.02	10.77 $\pm$ 0.32	327.1 $\pm$ 4.4	5.69 $\pm$ 0.16
Nakinyika	72	7.32 $\pm$ 0.22	2.78 $\pm$ 0.03	10.42 $\pm$ 0.21	292.9 $\pm$ 7.2	5.63 $\pm$ 0.11
Namwezi	54	7.81 $\pm$ 0.14	2.81 $\pm$ 0.04	11.04 $\pm$ 0.25	317.6 $\pm$ 7.0	6.02 $\pm$ 0.15
Nante	36	7.61 $\pm$ 0.25	2.93 $\pm$ 0.04	10.52 $\pm$ 0.22	264.8 $\pm$ 12.8	6.19 $\pm$ 0.22
Nassaba	66	7.43 $\pm$ 0.12	2.96 $\pm$ 0.02	11.02 $\pm$ 0.26	303.4 $\pm$ 10.3	5.65 $\pm$ 0.11
Ndibwabalangira	66	7.11 $\pm$ 0.23	2.84 $\pm$ 0.05	10.64 $\pm$ 0.39	288.1 $\pm$ 12.2	5.49 $\pm$ 0.19
Nfuuka	30	7.27 $\pm$ 0.17	2.85 $\pm$ 0.01	11.16 $\pm$ 0.18	296.5 $\pm$ 2.7	5.93 $\pm$ 0.21
Siira	36	7.64 $\pm$ 0.25	2.98 $\pm$ 0.03	13.35 $\pm$ 0.06	376.1 $\pm$ 7.0	5.97 $\pm$ 0.29
Tereza	42	6.90 $\pm$ 0.17	2.69 $\pm$ 0.02	10.78 $\pm$ 0.38	286.1 $\pm$ 5.6	5.69 $\pm$ 0.14
<b>Nakitembe clone set</b>						
Mbwazirume	54	7.48 $\pm$ 0.16	2.70 $\pm$ 0.04	10.94 $\pm$ 0.25	305.9 $\pm$ 6.1	5.74 $\pm$ 0.13
Nakitembe	48	7.73 $\pm$ 0.24	2.92 $\pm$ 0.04	11.68 $\pm$ 0.55	319.6 $\pm$ 6.2	5.55 $\pm$ 0.13
Nandigobe	48	7.81 $\pm$ 0.22	3.36 $\pm$ 0.05	12.00 $\pm$ 0.33	306.4 $\pm$ 9.1	6.67 $\pm$ 0.15
<b>Nakabululu clone set</b>						
Bukumu	24	8.13 $\pm$ 0.35	3.55 $\pm$ 0.08	8.08 $\pm$ 0.18	253.7 $\pm$ 11.2	7.92 $\pm$ 0.63
Kazirakwe	48	7.13 $\pm$ 0.21	2.04 $\pm$ 0.02	8.26 $\pm$ 0.22	288.0 $\pm$ 9.0	5.90 $\pm$ 0.15
Kibuzi	24	7.88 $\pm$ 0.31	3.01 $\pm$ 0.05	11.83 $\pm$ 0.47	320.3 $\pm$ 8.7	6.63 $\pm$ 0.20
Mukubakonde	24	3.21 $\pm$ 0.10	3.80 $\pm$ 0.14	6.98 $\pm$ 0.18	4.5 $\pm$ 1.1	4.71 $\pm$ 0.13
Nakabululu	36	7.33 $\pm$ 0.20	2.08 $\pm$ 0.01	9.29 $\pm$ 0.14	299.7 $\pm$ 2.1	5.89 $\pm$ 0.14
Nakasabira	36	6.94 $\pm$ 0.23	2.02 $\pm$ 0.02	9.49 $\pm$ 0.28	276.6 $\pm$ 7.5	6.08 $\pm$ 0.06
Nakayonga	24	7.00 $\pm$ 0.20	2.04 $\pm$ 0.02	8.73 $\pm$ 0.31	271.6 $\pm$ 10.5	5.88 $\pm$ 0.22
Nakyatengu	30	7.27 $\pm$ 0.30	2.17 $\pm$ 0.04	9.90 $\pm$ 0.42	306.4 $\pm$ 8.1	5.20 $\pm$ 0.19
Salalugazi	24	7.42 $\pm$ 0.31	2.60 $\pm$ 0.05	10.69 $\pm$ 0.46	300.6 $\pm$ 15.1	6.04 $\pm$ 0.22

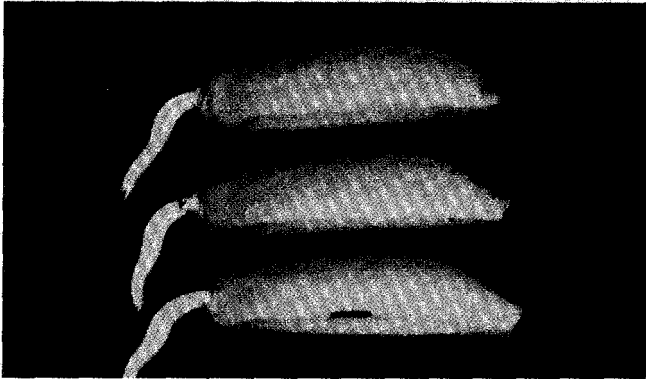


Plate 1. Pistils of a female sterile cultivar *Mukubakonde* showing long styles with pronounced longitudinal grooves, and vestigial stigmas.

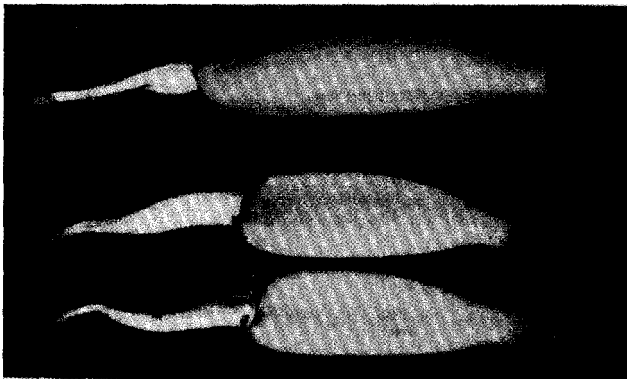


Plate 2. Pistils of a female sterile cultivar *Bukumu* showing long styles with wide style bases.



Plate 3. Pistils of *Kazirakwe*, a typical female fertile cultivar with short styles, normal size of the stigma and a small style base.

studies on seed set and conventional breeding in highland bananas.

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