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Reproductive efficiency and breeding potential of East African highland (*Musa* AAA-EA) bananas

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Abstract

The East African highland banana (*Musa* spp. AAA) is an economically important food crop. The crop is affected by a number of diseases and pests. Genetic improvement of the crop is hindered by very low seed set and poor seed germination. The objective of this study was to compare seed set, seed quality and embryo rescue rates of hybrid seeds obtained from 20 East African highland banana cultivars crossed with a fertile diploid species, *Musa acuminata* spp. *burmannicoides* 'Calcutta 4', as a male parent. There was great variation in seed set, seed quality and in vitro embryo germination rates among the cultivars. Although 72% (range = 47–88%) of the seeds appeared normal externally characterized by black hard integuments, only 59% (range = 35–81%) contained embryos, of which 9% (range = 0–22%) germinated. This study demonstrated that hard-seededness alone does not signify the presence of an embryo and should not be regarded as a measure of seed fertility in East African highland bananas. Cultivars 'Entukura', 'Enzirabahima' and 'Kabucuragye' of the 'Nfuuka' clone set were superior in terms of seed set, presence of seeds with embryos and culturability of embryos. These cultivars are recommended as female parents for a crossing program in the improvement of East African highland bananas. The low embryo rescue rates suggest that hybrid seeds derived from East African highland banana possess factors that cause high embryo abortion. This may be ascribed to endosperm breakdown, which can release toxins.
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Keywords: Breeding; Embryo culture; Fertility; *Musa*; Seed set

1. Introduction

The East African highland bananas (*Musa* spp. AAA) are an economically important crop in the Great Lakes region of East Africa, because they are the staple food ('matooke') and are also used to produce alcoholic and non-alcoholic beverages. The crop is

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susceptible to foliar diseases and pests that cause serious yield losses. These include black Sigatoka (*Mycosphaerella fijiensis*), nematodes (*Radopholus similis*, *Pratylenchus goodeyi*, *Helicotylenchus multicinctus*), viruses (banana bunchy top, banana streak badnavirus), the banana weevil (*Cosmopolites sordidus*) and bacterial wilt (*Xanthomonas campestris* pv *musacearum*). Adequate levels of resistance against these diseases and pests have not been found in the cultivated bananas but have been identified in wild species. Of particular interest is the wild diploid species Calcutta-4 (*Musa acuminata* subspecies *burmannicoides*) (De Langhe and Devreux, 1960), which is resistant to black Sigatoka and a number of other diseases and pests. 'Calcutta 4' with an AA genome composition is one of the progenitors of edible bananas and has been used in many breeding programs to transfer black Sigatoka resistance to cultivated bananas (Swennen and Vuylsteke, 1993; Vuylsteke et al., 1993; Rowe and Rosales, 2000). The East African highland bananas are triploid ($2n = 3x = 33$) and hence characterized by low male and female fertility that results in very low seed yield and germination rates (Simmonds, 1960; Bakry and Horry, 1992). However, embryo culture is reported to enhance seed germination and hybrid recovery. Embryo culture is the sterile isolation and in vitro growth of mature embryos with the goal of obtaining a viable plant (Pierik, 1987). Research on plantains has shown that it is possible to obtain embryo germination rates of up to 30% on phytohormone free medium compared to 1% from direct sowing of seeds in soil (Vuylsteke and Swennen, 1992; Ortiz and Vuylsteke, 1995). Thus, embryo culture is considered as an integral component of banana-breeding programs.

Conventional crossing methods have been successful in producing inter- and intra-specific hybrids as a result of minimizing infertility barriers. For example, desired traits can be introduced from one triploid to another through genetic bridging. Improvement of triploid *Musa* species has been achieved through crossing $3x$ landraces with $2x$ (diploids) wild or improved to produce $4x$ tetraploids that generally display greater male and female fertility. Selected tetraploids are then crossed with improved diploids to produce sterile secondary triploids (Pillay et al., 2002). Ultimately, the success in banana breeding relies on the identification of female fertile landraces that produce good rates of quality seeds. Therefore, this study was conducted with the objectives of: (i) evaluating hybrid seed quantity and quality and (ii) determining in vitro embryo rescue rates of hybrid seeds obtained from East African highland banana cultivars.

2. Materials and methods

2.1. Plant material and crosses

The East African highland bananas in Uganda are grouped into five clone sets, namely Mbidde, Musakala, Nakabululu, Nakitembe and Nfuuka (Karamura, 1998; Karamura and Pickersgill, 1999). However, in this study we considered the cultivars of Mbidde, Nakabululu and Nfuuka clone sets because of their high seed set. The study was conducted from 1996 to 1998 with hybrid seeds from crosses between 20 East African highland banana cultivars (female parent) and a fertile wild diploid banana *M. acuminata* spp. *burmannicoides* 'Calcutta 4' (pollen source) (Table 1). The plants were established in pollination blocks at Kawanda Agricultural Research Institute (Kawanda, Uganda, 0°25′N, 32°32′E) at 1195 masl. Male buds from which pollen was collected were covered with cotton bags to avoid contamination. Likewise, female inflorescences were bagged with transparent plastic bags from the time of shooting until after the last hand had been pollinated. Female flowers were considered ready for pollination when the bracts had opened halfway with fresh creamy white stigmas. Pollination was done between 07:00 and 10:00 h by opening the bracts of the female inflorescences to expose the stigmas and rubbing the anthers onto them. On average, one hand was pollinated per day until all hands in a bunch were completed. The pollinated bunches were harvested at first indications of ripening. Bunches were transferred to a ripening room and treated with ethylene gas to achieve uniform ripening. The pulp of the fruits was examined manually for seeds, which were extracted, washed and air-dried. The total number of seeds per bunch was recorded for each cultivar. In the laboratory, seeds were squeezed between the thumb and first finger to sort out seeds with hard black seed coats that were finally used for embryo culture.

Table 1
Means for seed set, proportion of hard black seeds, embryo presence and embryo germination of 20 East African highland banana cultivars

Cultivar	Clone set	No. of seeds handled	Seed set/bunch	Percentage		
				Hard black seeds[a]	Embryo recovery[b]	Embryo germination[c]
Enkara	Mbidde	68	5.8	68.4	62.0	21.2
Kazirakwe	Nakabululu	338	9.9	88.1	71.9	3.9
Nakabululu	Nakabululu	35	4.4	81.4	71.7	0.0
Nakasabira	Nakabululu	221	9.1	78.1	62.5	0.6
Nakyetengu	Nakabululu	92	8.0	79.0	64.9	13.5
Bitambi	Nfuuka	30	3.4	84.6	80.7	22.2
Entukura	Nfuuka	949	26.4	83.7	59.6	13.3
Enyeru	Nfuuka	173	15.7	62.6	57.2	0.7
Enzirabahima	Nfuuka	790	16.1	82.0	65.6	8.4
Kabucuragye	Nfuuka	434	14.3	77.2	60.0	13.8
Nabusa	Nfuuka	42	3.4	77.4	40.6	0.0
Nakabinyi	Nfuuka	134	6.0	81.1	66.4	8.8
Nakawere	Nfuuka	163	4.6	73.2	60.4	15.1
Nakayonga	Nfuuka	311	7.8	73.0	60.4	13.6
Namande	Nfuuka	115	7.1	64.3	48.9	7.6
Namwezi	Nfuuka	184	5.9	65.2	49.7	12.9
Nante	Nfuuka	185	10.9	53.2	54.0	0.5
Nfuuka	Nfuuka	106	5.1	46.7	34.7	13.7
Tereza	Nfuuka	265	7.9	68.7	57.3	4.5
Tuulatwogere	Nfuuka	99	5.5	47.2	51.4	2.9
Total		4734				
Mean			8.9	71.7	59.0	8.9
LSD 0.05			10.6	19.5	23.0	14.5
CV (%)			25.4	16.5	23.5	73.4

[a] Proportion of seeds with hard integument out of the total number of seeds handled.
[b] Proportion of seeds that had embryos out of the seeds with hard integuments.
[c] Proportion of germinated embryos out of all the extracted embryos.

2.2. Embryo culture

Embryo culture was done 3–10 days after seed harvest. The seeds were surface sterilized by dipping, consecutively, in 95% ethanol for 3 min, 15% (v/v) commercial bleach (NaOCl) solution containing 0.2% Tween-20 for 20 min, and rinsing three times with sterile distilled water. The seeds were cracked under sterile conditions to expose the white to cream mushroom-shaped embryo (Chin, 1996) that was extracted and cultured on Murashige and Skoog medium (Murashige and Skoog, 1962). The medium was supplemented with 0.4 mg/L thiamine–HCl, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine–HCl, 2.0 mg/L glycine, 20 mg/L ascorbic acid and 30 g/L sucrose. The pH of the medium was adjusted to 5.8 during preparation and solidified with 2.3 mg/L phytagel. Fifteen millilitres of the medium was dispensed into culture tubes and autoclaved for 15 min at 121 °C and 1.4 bars. The cultured embryos were initially incubated in the dark until germination and then transferred to lighted conditions (2000 lx) of 16 h at 28 ± 1 °C.

2.3. Data analysis

The number of seeds with hard black integuments was recorded and expressed as a percentage of total number of seeds. The percentage embryo recovery was derived from the proportion of hard black coated seeds with embryos. The number of germinated embryos was expressed as a percentage of the total embryos. The means for seed set per bunch and percentage embryo germination were subjected to log $(x + 1)$ and arcsine transformation (Gomez and Gomez, 1984; Compton, 1994), respectively. The

means for the 3 years were subjected to ANOVA using SAS (1999). Pearson's correlation coefficients were calculated to examine the relationship between number of seeds, proportion of seeds with hard black integuments, embryo recovery and germination.

3. Results and discussion

3.1. Hybrid seed set

The mean hybrid seed set varied greatly ($P = 0.014$) among the highland banana cultivars ranging from 3.4 in 'Bitambi' to 26.4 in 'Entukura' (Table 1). Since a common male parent was used in all the crosses, these results indicate that seed set is partly affected by the genotype of the female parent. Although seed set in bananas appear to be affected by seasons, the pollination done in this study was conducted throughout the year for 3 years. It is known that meiotic irregularities including lagging chromosomes and univalent formation can also influence fertility in *Musa* (Adeleke et al., 2004). The hybrid seeds appeared rounded with a flat top and bottom, characteristic of *Musa acuminata* (Chin, 1996) (Fig. 1). Seeds were either brown or black and varied widely in their level of development. Those with brown integuments were generally soft and empty probably due to abortion. Seeds with black integuments also varied in nature: (i) some were soft and empty; (ii) some had a large endosperm but no embryo and finally; (iii) some had normal endosperm and an embryo. The last two categories were hard hereafter termed 'hard black seeds'. The proportion of hard black seeds was substantially different among cultivars ($P = 0.001$) and ranged from 47% in Tuulatwogere to 88% in 'Kazirakwe'. Seventy-two percent of the 4734 seeds obtained in this study were hard black seeds (Table 1).

Fig. 1. East African highland banana (*Musa* spp. AAA): (A) black seeds with hard rough seed coats (9×); (B) opened seed before and after embryo extraction, top and bottom, respectively (60×); (C) seedling 10 days after embryo germination (2×).

3.2. Embryo culture

There were significant differences ($P = 0.001$) among cultivars in the proportion of hard black seeds with embryos. The values ranged from 35% for 'Nfuuka' to 81% for 'Bitambi' with an average of 59% (Table 1). These values were similar to those reported for the Popoulou/Maia Maoli subgroup of bananas (Bakry and Horry, 1992) and in plantains (Jenny et al., 1994). The absence of embryos in endosperm-filled seeds can be due to embryo abortion in the early stages of seed development or a disrupted embryo–endosperm relationship (Vuylsteke and Swennen, 1992). Cultivar differences in embryo germination rates was high ($P = 0.036$) with 'Bitambi' recording the highest rate (22%). Overall, 59% of the hard black seeds contained embryos of which only 9% germinated (Table 1). Apart from cultivar 'Enkara' and 'Nakyetengu' that belong to clone sets Mbidde and Nakabululu, respectively, cultivars with good germination belonged to the Nfuuka clone set. Generally, embryo germination took 8 ± 2 days compared to 14 days reported in plantains (Vuylsteke et al., 1990), indicating the suitability of the in vitro culture conditions used in our study.

There was no linear correlation between seed set, hard black seeds and the number of extractable embryos and embryo germination rates. However, there was a strong relationship between the proportion of the hard black seeds and number of recoverable embryos ($r = 0.605$, $P = 0.001$) and a rather weak association ($r = 0.329$, $P = 0.010$) between recoverable embryos and germination (Table 2). This implies that seed set is not a reliable measure of seed fertility in *Musa*. Rather female fertility should be based on hard-seededness, presence of embryos and the ability of the embryos to germinate.

On this basis, 'Entukura', 'Enzirabahima' and 'Kabucuragye' were the most female fertile cultivars as measured by the total number of seeds, proportion of hard black seed, presence of embryos and

Table 2
Correlation coefficients among total number of seeds, percentage hard black seeds, embryos and germination in 20 East African highland bananas

	Total seeds	Percentage of hard black seeds[a]	Percent embryo recovery[b]
Percentage of hard black seeds[a]	0.137		
Percent embryo recovery[b]	0.212	0.605*	
Percent embryo germination[c]	0.158	0.16	0.329**

[a] Proportion of seeds with hard integument out of the total number of seeds handled.
[b] Proportion of seeds that had embryos out of the seeds with hard integuments.
[c] Proportion of germinated embryos out of all the extracted embryos.
* Significant at 0.1% level.
** Significant at 1% level.

germination. These cultivars are recommended as female parents for the genetic improvement of the East African highland bananas. In contrast, cultivars 'Bitambi', 'Enkara', 'Nakabinyi', 'Nakawere', 'Nakayonga', 'Nakyetengu', 'Namande', 'Namwezi', 'Nfuuka' and 'Tereza' had poor seed set, but superior embryo germination. Success in improving these cultivars may depend on extensive hand pollination to obtain a sizable seed number. Although cultivars 'Enyeru', 'Kazirakwe', 'Nakasabira', 'Tereza' and 'Nante' showed relatively good seed set rates and embryo presence, they had very low rates of embryo germination.

Embryo-culture technique is important in the elimination of seed germination inhibitors localized in endosperm and seed coat (Pierik, 1987; Hartmann et al., 1990). However, the failure of most embryos to germinate even after the removal of restrictive barriers suggests that other factors may influence embryo germination in highland bananas. It is known that endosperm breakdown in *Arachis* releases toxins that influence embryo abortion (Pattee and Stalker, 1992). The germination rates observed in the highland bananas were greatly variable and lower than the highest rates reported for plantains (Vuylsteke et al., 1990; Ortiz and Vuylsteke, 1995). This could partly be attributed to cultivar and genome effects. Banana varieties show varying degrees of seed dormancy and respond differently to various dormancy breaking treatments (Chin, 1996). For example, some seeds have been reported to germinate readily after harvesting showing no sign of dormancy, but become dormant after drying; while others never germinate when freshly harvested, but germinate after drying (Chin, 1996).

By using embryo culture, a higher number of seedlings were obtained from hybrid seeds of the East African highland bananas than via normal soil germination that gave 1.4% seedling recovery (Talengera et al., 1996). Embryo culture is, therefore, an efficient tool for improving seedling yield in wide crosses in *Musa* that generally produces very low numbers of germinative seeds. Another advantage of the in vitro technique is that many identical banana plants can be produced from a single hybrid seedling by shoot tip culture. This enables evaluation of the hybrids in multiple locations since bananas generally show strong genotype × environment interactions (Vuylsteke et al., 1997).

Wide hybridization followed by embryo culture is a viable way of introgressing desirable traits from wild species into cultivated bananas. It also provides a means of widening the narrow genetic base of the East African highland bananas (Pillay et al., 2001) and offering a significant amount of genetic diversity for further improvement of the crop (Pillay et al., 2004).

4. Conclusions

Seed set, seed quality and embryo germination in the East African highland bananas was highly cultivar-dependent. Female fertility was greatly determined by a combination of embryo presence and subsequent embryo germination of the hybrid seeds. Highland bananas were substantially similar to plantains in seed quality but low in embryo germination. Cultivars 'Entukura', 'Enzirabahima' and 'Kabucuragye' were more promising female parents to use in the genetic improvement of the highland bananas.

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