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Development and functioning of the embryo sac in four triploid banana cultivars

Abstract – The objective of this work was to investigate the causes of sterility in a new set of triploid banana (*Musa* spp.) cultivars and to assess the chances of obtaining some progenies by manual cross-pollination. The developmental stages of female gametophyte were histologically recorded in ovules of four distinct triploid banana cultivars. Samples were taken on the day of flower opening and pollination and three days later. Morphologically mature embryo sacs were very rare in the two clones of the 'Cavendish' subgroup and in 'Prata Anã'. In 'Mysore', it occurred at a variable and low frequency. In 'Terrinha', it was extremely common. In the last two cases, the embryo sac maturation process was clearly continuing between the sampling days of flower opening and three days later. No pollen tube was positively identified in pollinated flowers within the integuments of any ovule of the older explants. Different proportions of mature embryo sacs are observed among the studied triploid genotypes of banana. The presence of mature embryo sacs is not an indication of functional capacity. Other causes of infertility in cultivated bananas can be due to some event immediately before or after fertilization, for instance, pollination tube growth failure or seed abortion.

Index terms: *Musa acuminata*, *Musa balbisiana*, plant breeding, sexual reproduction, female infertility.

Kenneth Shepherd[†],
Jorge Luiz Loyola Dantas[1],
Marie-Edith Goutant-Bakry[2],
Camille Ulrich Dzokouo Dzoyem[3] and
Frédéric Bakry[2] ✉

[1] Rua B, nº 145, Loteamento Vila Alzira, Santa Cruz, CEP 44380-000, Cruz das Almas, BA, Brazil.
E-mail: jorgeloyola1@gmail.com

[2] 4 Avenue des Chasseurs, 34130, Saint-Aunès, France.
E-mail: mgoutantbakry@gmail.com, fredbakry@gmail.com

[3] Institut de Recherche Agricole pour le Développement, BP 13 Njombé, Cameroon.
E-mail: camilledzoyem@gmail.com

[†] *In memoriam*

✉ Corresponding author

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Desenvolvimento e funcionamento de sacos embrionários em quatro cultivares triploides de banana

Resumo – O objetivo deste estudo foi investigar as causas da esterilidade em um novo conjunto de cultivares triploides de banana (*Musa* spp.) e avaliar as possibilidades de obter progênies por meio de polinização manual. Os estados de desenvolvimento do gametófito feminino foram histologicamente observados em óvulos de quatro cultivares de banana triploide. As amostras foram colhidas no dia da abertura das flores e da polinização e três dias depois. Os sacos embrionários morfologicamente maduros mostraram-se raros em dois clones do subgrupo 'Cavendish' e em 'Prata Anã'. Em 'Mysore', ocorreu a uma frequência variável e baixa. Em 'Terrinha', foi extremamente comum. Nos dois últimos casos, o processo de maturação dos sacos embrionários claramente continuava entre o dia da amostragem na floração e os três dias depois. Nenhum tubo polínico foi identificado em flores polinizadas nos integumentos de qualquer óvulo nos explantes mais velhos. Diferentes proporções de sacos embrionários são observadas entre os genótipos de bananas triploides. A presença de sacos embrionários não indica capacidade funcional. Outras causas de infertilidade em bananas cultivadas podem ser em razão de algum evento imediatamente antes ou depois da fertilização, como ausência de cariogamia ou aborto das sementes.

Termos para indexação: *Musa acuminata*, *Musa balbisiana*, melhoramento de plantas, reprodução sexuada, esterilidade feminina.

Introduction

Every year, approximately 140 million tons of bananas are produced in tropical and subtropical regions around the world (Lescot, 2020), they belong to the *Musa* genus and are often mistakenly referred to as trees, but they are actually giant monocotyledonous herbs with 2 to 9 m tall. Their cultivars are virtually sterile, being reproduced through vegetative propagation via suckers, with origin in two diploid wild species from Southeast Asia: *Musa acuminata* Colla (AA) and *Musa balbisiana* Colla (BB), which are the origins of the main diploid and triploid cultivated groups: AA, AB, AAA, AAB, and ABB (Simmonds & Shepherd, 1955; Bakry et al., 2009; Perrier et al., 2009).

Because global banana cultivation is restricted to a narrow genetic base (Bakry et al., 2020), there is an urgent need to expand the current cultivars with new genotypes resistant to pests, diseases, and adapted to climate change. However, genetics and sterility of bananas and plantains compromise the development of new varieties using hybridization (Heslop-Harrison & Schwarzacher, 2007; Chang et al., 2019), because seed production is often scarce, compromising pollination efforts to obtain new hybrids, since seed production is an essential requirement for genetic improvement through cross-breeding (Bakry et al., 2020).

The sterility of edible bananas occurs at three different stages (Menendez, 1958): deficient sporogenesis, as a result of an irregular meiosis in triploids; failed fertilization; and failed post-fertilization, although zygotes and seeds are developed, they produce embryos that do not germinate in their environment.

There are few studies concerning *Musa* spp. on the development of female gametophyte at any ploidy level and on the efficiency of fertilization after haploid pollination. Dodds (1945) first described the general course of female development in diploids of *Musa acuminata* (AA), from macrospore mother cell to mature embryo sac. The functional sacs have a characteristic bell shape with a chalazal pit, and the eight nuclei are organized into a micropylar apparatus consisting of: one oocyte; two synergids; three chalazals, which degenerate into the basal pit; and two polars close to the basal pit.

Shepherd (1954) first reported quantitative data from 'Gros Michel' (AAA group) in triploid cultivars. The author found that the frequency of occurrence of ovules with mature sacs at the time of flower opening varied from 9.8% to 18.0%, depending on where the samples were collected. However, many embryo sacs were abnormal in terms of nuclei number or arrangements. Later, Simmonds (1960) examined ovules of 'Mysore' (AAB group), 'Bluggoe' and 'Awack Legor' (both ABB group), and no embryo sac of mature aspect was found in 'Mysore' or 'Bluggoe'. It was observed in 'Bluggoe' that the number of nearly mature forms increased with the age of the flower. In 'Awack Legor', some mature sacs were present at two days before flower opening. All these studies are not recent, so new ones on new groups of cultivars are required.

The objective of this study was to investigate the causes of sterility in a new set of triploid banana cultivars and to assess the chances of obtaining progenies through manual cross-pollination.

Materials and Methods

This study is the result of a collaborative project between Brazil and France, through Empresa Brasileira de Pesquisa Agropecuária (Embrapa) and French Agricultural Research Centre for International Development (CIRAD), which was carried out in the mid-1980s and led by Dr. Kenneth Shepherd at Embrapa Mandioca e Fruticultura.

Quantitative data of the occurrence of mature embryo sacs at anthesis were obtained for four important triploid cultivars of the groups AAA, with one Nanica and one Nanicão plants of the Cavendish subgroup, and AAB, with three Mysore, two Terrinha, and one Prata Anã plants, of the subgroups Mysore, Plantain and Pome, respectively. Nanica is related to Dwarf Cavendish, Nanicão is close to the cultivar Valery in its characteristics, and Terrinha is a medium-height clone of French Plantain.

The plants were grown in the active banana germplasm bank at Embrapa Mandioca e Fruticultura, in the municipality of Cruz das Almas, in the state of Bahia, Brazil (12°39'S, 39°06'W, at an altitude of 225 m), with Af climate, according to Köppen's classification.

Samples were collected on the day of flower opening and pollination (day 0) and three days later (day 3). Pollination was carried out using the male-fertile wild *Musa acuminata* accession 'Long Tavoy'.

Quantitative data on embryo sacs as observations of pollen tube growth were obtained at Embrapa in 1987.

At that time, few sharp enough images were obtained. In Montpellier, at CIRAD, interferential contrast techniques, based on Herr's protocol, were used to better illustrate the images obtained back in 1987 in Brazil.

To prepare the samples, ovaries were stripped on the outer skin and thin inner skin, enclosing each of the three carpels. The axile placentation of ovaries in the genus greatly facilitates the excision of samples. All the ovules are relatively aligned at right angles to the central core. Pieces of 1.0 to 1.5 cm were taken from the apical and basal regions of the ovary. In order to preserve the tissues, the pieces were fixed for 24 hours using a solution containing equal parts (v/v) of Craf 1 and Craf 2 fixatives mixed immediately before use. Then, the material was rinsed for 24 hours in running water, after that, it was dehydrated in a graded series of six solutions of ethanol in increasing concentrations, from 20 to 100% being kept in each bath for 2 hours. The material was transferred to n-butanol in two successive baths, for 1 hour each bath. Then, infiltration was accomplished immersing the material in paraffin wax at melting point for 1.5 and 5 hours, using a conventional oven at 60°C.

The next step was the microtome sectioning and the mounting of ribbons on microscope slides. It was used an aqueous adhesive solution of gelatin followed by the extension of the ribbons on an electric hot plate. The material was dried for, at least, 48 hours at room temperature. The wax was removed immersing the slides in xylol for 20 min, then in ethanol, and later in distilled water. As a simplified procedure for preparation of samples for microscopy, temporary mountings were made using microscope cover glasses in a 0.1% lactophenol cotton blue solution made of equal parts of lactic acid, phenol, glycerol, and distilled water.

Although literature suggests samples of 30 µm thickness sections, in the present study, it was not possible to obtain enough adhesion of sections of ribbons with thickness greater than 20 µm. The authors believe it was due to wax quality or the microtome. This problem resulted in a substantial loss of information of the nuclear contents of the later developmental stages.

To better illustrate ovule development, it was used in complement a clearing-squash technique. Banana ovules were prepared using a procedure of Levieil & Huyghe (1985). They simplified the methodology developed by Herr (1971) for observations of megagametophytes of different plant species. Dessauw (1988) was the first one to use Herr's methodology with seeded and edible diploid bananas. The procedure has two steps: the fixation of the whole ovule and later removal of the carpels, and the observation of the cleared ovules using differential interference contrast, also known as Nomarsky's interference contrast. Carpels were removed from the fruits at anthesis, when ovules were still attached to the placenta. The ovules were fixed using FPA50 (formalin 40%, propionic acid, ethanol 50%; volumes ratio of 7:3:90) for 24 to 48 hours. After fixation, it was rinsed using ethanol 100% and stored in ethanol 70% for several months.

To study the fixed ovules, they were previously prepared for observation, immersing them for 30 min in lactic acid for clarification. After clarification, the ovules were dissected using binoculars. For microscopic examination, the nucelli was extracted from the integuments and put longitudinally on special slides with a small amount of Herr's fluid, modified to be harmless, phenol-free and xylene-free. The fluid consisted of a mixture of lactic acid 85%, chloral hydrate, and clove oil (1:1:1 w/w). For microscopy observation, a special slide arrangement helped to avoid crushing the nucelli and the embryo sacs. The slide and the cover slip were separated using two cover slips glued to the slide, framing the nucelli immersed in Herr's fluid.

The classification of the different developmental stages of the embryo sacs were based on the structures found, and six stages named as A1, A2, B1, B2, B3 and C were identified (Figures 1 and 2). Stage A1 presents total collapse of the mother cell or the megaspore; A2 shows some elongation of the megaspore, but it does not reach the palisade; B1, the megaspore shows elongation as far as the palisade, but without lateral growth, when it would be present two to four nuclei; B2 shows around 30 µm of lateral enlargement in the diameter of the micropylar, which would be equivalent to four to eight nuclei, but without migration of the nuclei to their final location; B3, it's observed apical enlargement to about 50 µm in diameter, sometimes with nucellar collapse adjacent to the sub-basal region (Figure 2); and C, embryo sac presents its mature form, with size and precise shape, depending of the cultivar (Figure 3). However, this mature form was never strictly a structure, since most of its lateral extension

towards the base arose from degeneration of the outer wall and of the adjacent nucellus.

Embryo sac distributions between two varieties was analyzed using the two-sample Kolmogorov-Smirnov test. This test verifies whether two samples come from the same distribution and makes a two-by-two comparison of data arranged as a function of an evolutionary process (Chakravarti et al., 1967). In the present study, the evolutionary process is the megasporogenesis. The significance level used was $\alpha = 0.01$.

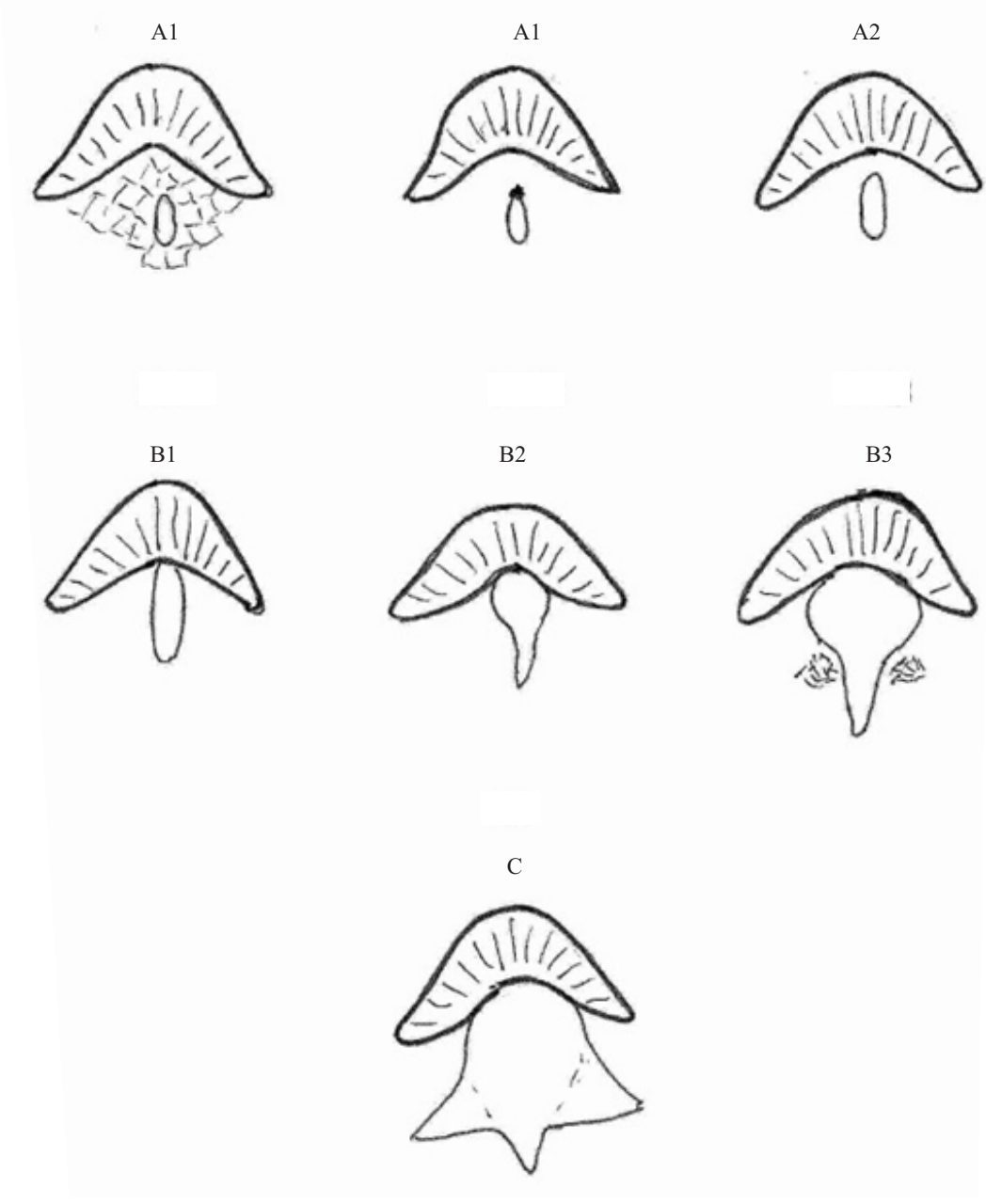

Figure 1. Morphological development stages of embryo sacs in *Musa* spp., showing total collapse of the mother cell or the megaspore (A1), megaspore elongated (A2), extension of the megaspore to the palisade (B1), lateral enlargement of the micropylar part of the megaspore (B2), continued apical enlargement and beginning of nucellar collapse adjacent to the sub-basal region (B3), and bell-shaped mature embryo sac (C).

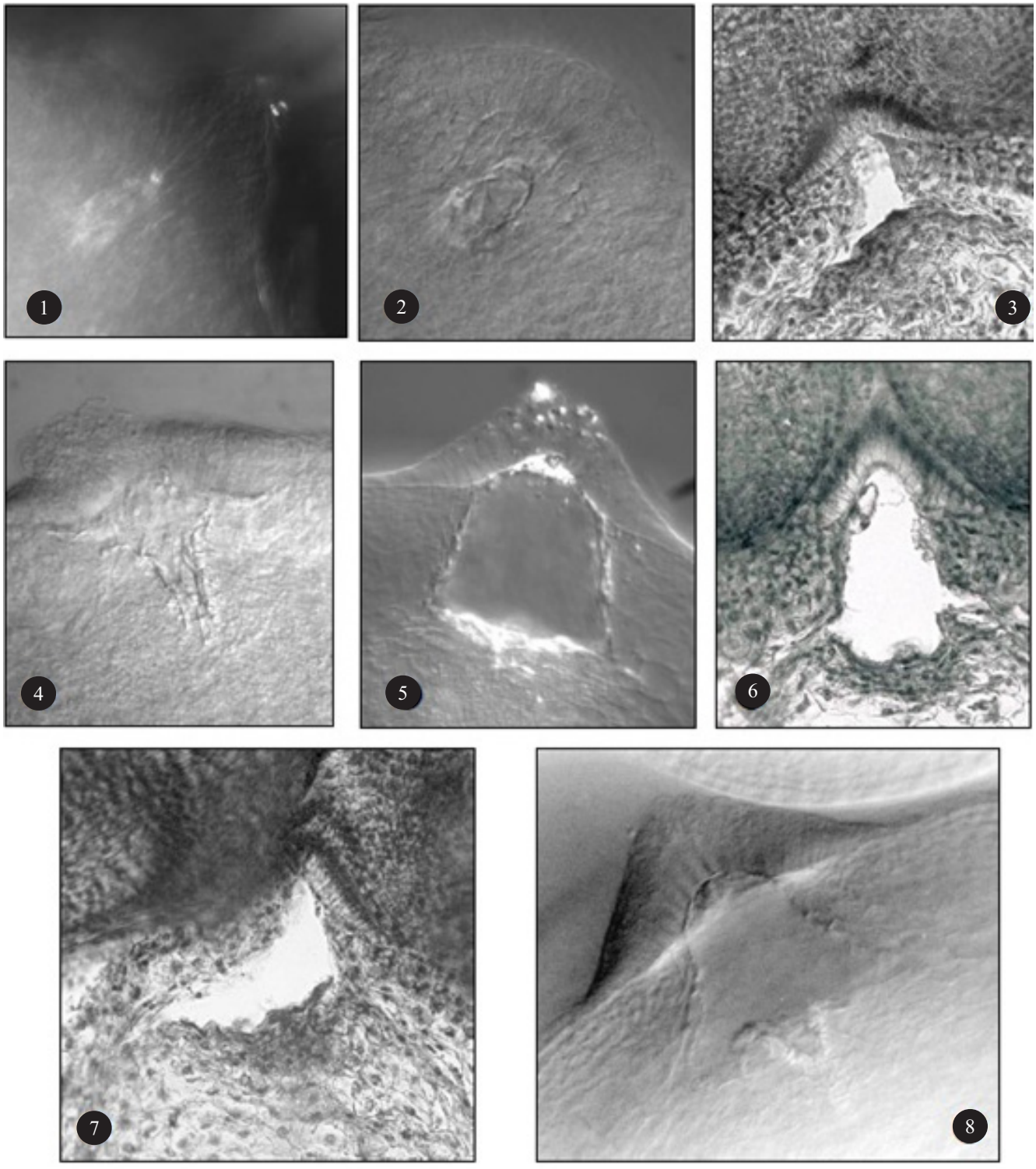

Figure 2. Images of the embryo sac development in the banana cultivars Nanica* at late stage A1 (1), Terrinha* at stage A2 (2), Nanica** at stage B2 (3), Terrinha* at stage B2 (4), Terrinha* at stage B3 (5), Nanica** at stage B3 (6), Mysore** at stage C showing mature half-sac (7), and Terrinha* at stage C showing mature and perfect embryo sac (8). (*)DIC observations. (**)Classical histology.

Results and Discussion

Along the observations, discrimination between stages A1 and A2 was only possible in samples at anthesis (day 0). In older samples (day 3), the occurrence of A1 was more frequent than A2, what was an unexpected result, consequently, in older samples, the stages A1 and A2 were considered as one single stage (A1+A2).

The possible effect of ovule position on embryo sac maturation in the ovaries was investigated. On day 0, the ovary apical part, the upper half close to the style of the flower, and the basal part, the lower half of the organ, were observed for all samples. For all clones on day 0, comparison of the distributions showed no significant differences in the development of the embryo sac at the basal part or the apical part of the ovaries (Table 1). For this reason, subsequent analyses

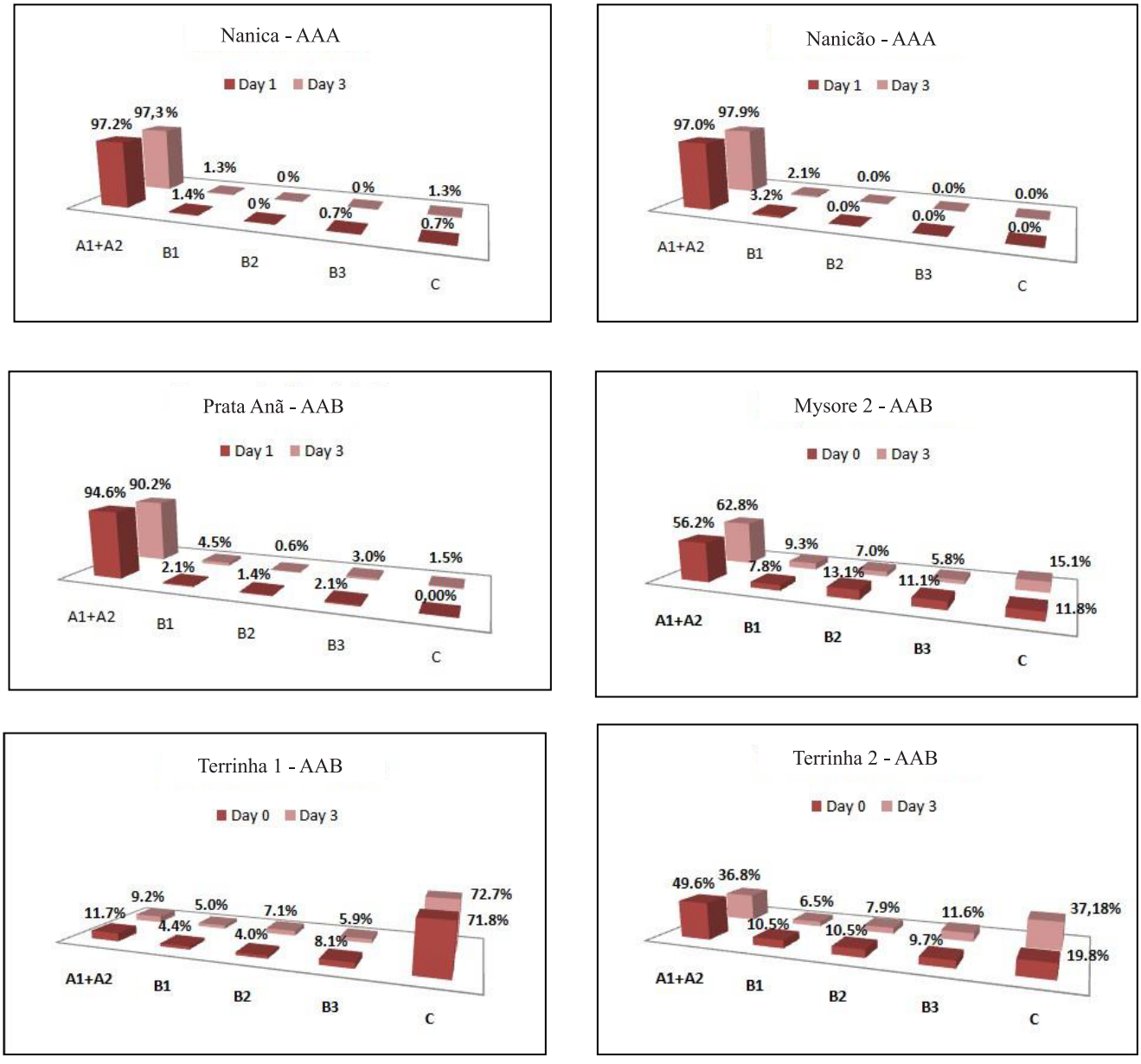

Figure 3. Schematic representation of embryo sac development in different triploid banana cultivars.

in this study were carried out by pooling the results obtained from the apical and basal parts at day 0 and this issue was not investigated at day 3.

In 'Cavendish' subgroup (AAA), 561 ovules from single inflorescences of clones of 'Nanica' and 'Nanicão' were examined (Table 1 and Figure 3), of which 15 ovules (2.7%) had developed beyond stage A2. There was no suggestion that this frequency was modified because of the day of sampling. Three apparently mature embryo sacs were found in the 'Nanica' material, of which, two, observed on day 3, were quite slender sacs, with diameters of 70 and 40 µm. In the 70 µm one, a sound-looking polar nucleus was located along with indications of a micropylar apparatus, which may have been a viable and normal sac. In the 40 µm one, two degenerated polars were found, but no evidence of a pollen tube was found on day 3. These results may indicate that the known female sterility of the subgroup (Simmonds, 1962) can be explained primarily due to the lack of mature and viable embryo sacs, independently whether the fertilization process occurred.

In 'Prata Anã' (AAB), 280 ovules were classified (Table 1 and Figure 3), and 21 of them had passed stage A2, which represented 5.4% of ovules on day 0 and 9.8% of ovules on day 3. The formula used to calculate the percentages on each day was: $1 - (A1+A2) / Total_{A+B+C}$. Two mature embryo sacs were

Table 1. Stages of *Musa* spp. embryo sac development as number of observations for each stage[(1)].

Cultivar (subgroup)	Ovule region	Day	Developmental stage							Total (A+B+C)	Group[(2)]
			A1	A2	A1+A2	B1	B2	B3	C		
Nanica (AAA)	Apical	0	56	10	66	1	0	0	0	67	a
	Basal	0	45	27	72	1	0	1	1	75	a
	Total	0	101	137	138	2	0	1	1	142	a
		3	-	-	146	2	0	0	2	150	a
Nanicão (AAA)	Apical	0	66	26	92	4	0	0	0	96	a
	Basal	0	23	8	31	0	0	0	0	31	a
	Total	0	89	34	123	4	0	0	0	127	a
		3	-	-	139	3	0	0	0	142	a
Prata Anã (AAB)	Apical	0	14	56	70	2	1	1	0	74	ab
	Basal	0	18	51	69	1	1	2	0	73	ab
	Total	0	32	107	139	3	2	3	0	147	ab
		3	-	-	120	6	1	4	2	133	ab
Mysore 1 (AAB)	Apical	0	47	72	119	29	9	6	4	167	c
	Basal	0	34	45	79	9	10	5	1	104	bc
	Total	0	81	117	198	38	19	11	5	271	c
		3	-	-	204	44	18	23	24	313	c
Mysore 2 (AAB)	Apical	0	29	17	46	6	7	9	8	76	bd
	Basal	0	20	20	40	6	13	8	10	77	de
	Total	0	49	37	86	12	20	17	18	153	bd
		3	-	-	108	16	12	10	26	172	bd
Mysore 3[(3)] (AAB)	Apical	0	27	25	52	20	4	1	0	153	bc
	Basal	0	30	24	54	26	3	1	0	84	bc
	Total	0	57	49	106	46	7	2	0	161	bc
		3	-	-	-	-	-	-	-	-	
Terrinha 1 (AAB)	Apical	0	10	4	14	9	7	6	90	126	f
	Basal	0	12	9	21	4	5	18	124	172	f
	Total	0	22	13	35	13	12	24	214	298	f
		3	-	-	31	17	24	20	245	337	f
Terrinha 2 (AAB)	Apical	0	24	34	58	12	16	7	26	119	de
	Basal	0	28	37	65	14	10	17	23	129	de
	Total	0	52	71	123	26	26	24	49	248	de
		3	-	-	102	18	22	32	103	277	de

[(1)]Means followed by equal letters, in the rows, do not differ significantly in their distribution. [(2)]Result of the two-sample Kolmogorov-Smirnov's test, $\alpha=0.01$. [(3)]There are no data of total number of observations of 'Mysore' 3 on day 3.

found on day 3: C / $Total_{A+B+C}$ = 1.5%, with diameters of 100 and 200 µm. One of the mature embryo sacs may have been fertilized, since a structure resembling a pollen tube was seen at the micropylar and the cytoplasm was darkened, which suggests fertilization (Bouharmont, 1961). It was seen one probable female nuclei, two micropylars, and two polars. Additionally, one of the four ovules showing B3 stage on day 3 had much darker cytoplasm. In an unusual large sac, only one single polar nucleus remained. These observations were unclear and correct interpretation was uncertain. Again, the primary difficulty in obtaining hybrid seeds from this triploid parent may be the scarcity of mature and viable embryo sacs.

In 'Mysore' (AAB), slides of day 0 samples from three bunches were analyzed, along with slides of day 3 ovules from two bunches (Table 1 and Figure 3). A total of 1,070 embryo sacs were observed, of which 66% had not passed development stage A2. This percentage was similar in the three bunches and did not vary in terms of part of the ovary or sampling day. However, the frequency of observations reaching stage C was variable between bunches, in contrast to the observations made by Simmonds (1960), the proportion of stage C in the present study increased with the age of the ovaries. Different from 'Cavendish' clones, 'Mysore' presented ongoing development of the embryo sacs from stage A1+A2 to stage C, and no pollen tube was observed in older samples on day 3. The number of embryo sacs observed was 73, and their diameters ranged from 60 to 180 µm. One of the smallest morphological developments was markedly one-sided (Figure 3). It was not possible to classify the degree of morphological normality of embryo sacs due to the uncertainty in the interpretation of the observations. One embryo sac was apparently complete and normal in terms of nuclear content and distribution, but the others presented traits of abnormal nuclear content or necrotic degeneration.

In 'Terrinha' (AAB, 'Plantain' subgroup), the two bunches studied presented promising results in terms of number of ovules beyond stage A2 and number of embryo sacs achieving mature form (Table 1 and Figure 3). In bunch 1, more than 90% of the total ovules presented some development and more than 71% of the total ovules contained mature embryo sacs, stage C, on day 0 (71.8%) or day 3 (72.7%). For bunch 2, the numbers were 57% and 29%, respectively, and fully developed embryo sacs were more common in ovules on day 3 than on day 0, which was almost twice as more frequent.

On day 0, bunch 1 of Terrinha presented 81 embryo sacs in apical ovules and 81 in basal ovules, with diameter ranging from 80 to 280 µm, present in four to 14 successive sections. The mean diameter of apical embryo sacs was higher than the basal one. As other clones, morphological details were not always visible, but it could be observed 24 embryo sacs apparently normal in number and form of nuclei. On the other hand, there was an excessive number of polar nuclei and a displacement towards apical position, additionally, some embryo sacs were clearly necrotic. On day 3, 50% of the ovules located in the apical part of the ovaries showed embryo sacs as enormous cavities occupying one third or more of the nucellus (Figure 2). One embryo sac presented sufficient details to confirm a normal nuclear set. A considerable number of embryo sacs seemed to have a degenerated apical egg apparatus formed by the oocyte and the two synergids. Enlarged embryo sacs were found in 13% of the basal ovules, where details of nuclear organization could not be seen.

For bunch 2 of 'Terrinha', on day 0, two of the 26 apical embryo sacs presented a large hole in the nucellus, but none of the basal ones did. Normal diameters of the embryo sacs were from three to 11 sections, one embryo sac presented a single lateral horn, three narrow embryo sacs were quite elongated, one of these narrow embryo sacs seemed to have a perfect five-nucleate form, and one embryo sac presented a sound-looking egg apparatus. On day 3, a large nucellar hole was observed in five out of 59 apical embryo sacs and one out of 44 basal embryo sacs, and two of the undamaged apical ovules may have had the proper nuclear composition.

In 'Terrinha' bunches, no pollen tube was seen in any section of the vicinity of the micropylar or integuments. An appreciable number of embryo sacs available in both bunches were capable of being successfully fertilized, whatever the ploidy of the zygotes generated, either in themselves or in relation to the ploidy of the endosperm.

Despite the problems to investigate the causes of sterility in the triploid banana genotypes in the present study, it was possible to extend the knowledge about their capacity to yield morphologically mature embryo

sacs. For instance, all the four genotypes studied presented some embryo sacs in a mature form, which were extremely rare in 'Cavendish', rare in 'Prata Anã', uncommon in 'Mysore' and common in 'Terrinha'. All of them are potential female parents for hybridization purpose.

A surprising result was that the studied clones apparently carried more functional embryo sacs than the seeds they usually produce in controlled hybridizations. It was estimated that there are at least 20,000 ovules potentially fertilized at anthesis. This calculation is based on a single triploid ovary of AAA or AAB genotypes containing more than 300 ovules (Fortescue & Turner, 2011), in bunches with more than five hands and 14 fruits per hand. Based on the results of the present study, considering only the developmental stage C, it is possible to obtain at least 100 seeds per pollinated bunch of 'Cavendish' and up to 14,000 seeds of 'Terrinha' (Table 2). In this case, even a low frequency of functional and genetically balanced embryo sacs should be sufficient to produce useful seed yields, which, in practice, is not the case. 'Cavendish' is known to be practically sterile (Simmonds, 1962), although seeds have been obtained more recently through intensive efforts (Aguilar Morán, 2013). Similarly, hybrids have been obtained from clones of the 'Prata' group (Shepherd et al., 1994). However, there are few hybrids compared to the high proportion of mature embryonic sacs found in this study.

Due to the results obtained for 'Terrinha', it was expected that seed production would be prolific in this plantain, but this was not observed. If other plantains exhibit similar ovarian behavior, it would be possible that a wider range of genotypes within this subgroup could serve as female parents than it is currently found (Bakry & Horry, 1992; Vuylsteke et al., 1993). However, these results suggest that the presence of embryo sacs is not an indication of functional capacity. Another hypothesis is that the environment affects the proportion of embryo sacs produced (Shepherd, 1954; Waniale et al., 2021). It is also likely that infertility in plantain may can be caused by some event immediately before or after fertilization (i.e., seed abortion), among others. Therefore, two main causes of abortion can be suggested: a genetic or chromosomal imbalance, especially between the zygote and the endosperm, at least at the triploid level; and an inhibitory or

Table 2. Estimated number of seeds per pollinated bunch based on percentage of C-stage for each clone of *Musa* spp.

Cultivar (subgroup)	Day	Total ovule	A1+A2		C-stage		Estimated number of seed per bunch[(1)]
			Number	%	Number	%	
Nanica	0	142	138	97.2	1	0.7	141
(AAA)	3	150	146	97.3	2	1.3	267
Nanicão	0	127	123	96.9	0	0.0	0
(AAA)	3	142	139	97.9	0	0.0	0
Prata Anã	0	147	139	94.6	0	0.0	0
(AAB)	3	133	120	90.2	2	1.5	301
Mysore 1	0	271	198	73.1	5	1.8	369
(AAB)	3	313	204	65.2	24	7.7	1,534
Mysore 2	0	153	86	56.2	18	11.8	2,353
(AAB)	3	172	108	62.8	26	15.1	3,023
Mysore 3[(2)]	0	161	106	65.8	0	0.0	0
(AAB)	3	-	-	-	-	-	-
Terrinha 1	0	298	35	11.7	214	71.8	14,362
(AAB)	3	337	31	9.2	245	72.7	14,540
Terrinha 2	0	248	123	49.6	49	19.8	3,952
(AAB)	3	277	102	36.8	103	37.2	7,437

[(1)]Minimum number of ovules per bunch used for estimative is 20,000. [(2)]There are no data on 'Mysore' 3 on day 3.

competitive effect on embryo development resulting from parthenocarpic fruit development in relation to auxin metabolism (Simmonds, 1953; Menendez, 1958). It is for this reason that in vitro embryo rescue has been introduced as an aid to banana breeding (Bakry, 2008; Ongagna et al., 2020).

To fertilize these plants, pollen should land on a compatible stigma, adheres, hydrates, and germinates to form the pollen tube, a structure specialized to carry sperm cells to the ovule (Sundberg & Østergaard, 2009). In the present study, pollen tube reaching the embryo sacs was an extremely rare to be observed, possibly because of failures due to stigma immaturity at anthesis, but may also be the result of delayed, inhibited, or limited penetration of normal styles, one of the major limitations to seed set.

Shepherd (1954, 1960) found deficiencies in pollen tube growth, resulting in delayed penetration or its cessation at, or near to, the style base, limiting penetration into the ovary. Later, the same author also observed another anomaly within the ovary apex. It has also been shown that ovules of edible triploids exhibit a micropylar exudate in both A and B genomes. This exudate may have some effect on pollen tube penetration of embryo sacs (Fortescue & Turner, 2005).

More recently, other authors have suggested that phenolic compounds and oxidative enzymes near the septal nectaries of female flowers may also limit pollen tube growth in 'Grande Naine' ovaries (Silva et al., 2021, 2022), a plant of the 'Cavendish' subgroup. Therefore, the reasons for the failure of pollen tube growth can be many and are as a result of the domestication process of cultivated bananas, since these barriers are not observed in wild bananas.

Conclusions

1. Different proportions of mature embryo sacs are observed between the studied triploid genotypes of banana (*Musa* spp.).

2. The presence of mature embryo sacs is not an indication of functional capacity.

3. Other causes of infertility in cultivated bananas can be due to some event immediately before or after fertilization, for instance, pollination tube growth failure or seed abortion.

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