PAB
Pesquisa Agropecuária Brasileira

Genetics/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Suppression of female flowers and pollen fertility of male flowers of banana plants

Abstract – The objective of this work was to evaluate the effect of the suppression of female flowers on the pollen fertility of male flowers of banana plants. Two modes of female flower suppression, one partial and one complete, were applied shortly after flowering to diploid, triploid, and tetraploid banana genotypes. The effect of flower suppression on the number of male flowers and the length of their anthers was evaluated, as well as pollen viability and the size and number of pollen grains per anther. Pollination tests were performed to evaluate the number of seed obtained from the progeny of some genotypes. The total suppression of female flowers significantly increases pollen fertility, estimated by an increase in the number of pollen grains per anther (up to 80% per anther). Total female flower suppression also improves significantly seed production in the crosses (from 35 to 160% per pollinated bunch), which suggests a positive influence of the sap flowing through the bunch on the efficiency of banana pollen.

Index terms: *Musa*, genotype, hybridization, pollen inefficiency, sap flow, seed.

Camille Ulrich Dzokouo Dzoyem[1] ✉, Lucien Ibobondji Kapuku[2], Zenabou Ndiang[3], Gaetan Romaric Ngapmeu Tchabong[3], Emmanuel Youmbi[4] and Frédéric Bakry[5]

[1] Institute of Agricultural Research for Development, Njombe Multipurpose Station, PO Box 13 Njombe, Cameroon. E-mail: camilledzoyem@gmail.com

[2] Institut National pour l'Étude et la Recherche Agronomiques, Research Station of Kinzau, PO Box 2037 Kinshasa, Democratic Republic of Congo. E-mail: ibobondji@gmail.com

[3] University of Douala, Faculty of Sciences, Plant Biology and Physiology Laboratory, PO Box 24157 Douala, Cameroon. E-mail: ndizena@yahoo.fr, ngapmeugaetan@gmail.com

[4] University of Yaoundé I, Faculty of Sciences, Department of Plant Biology, Laboratory of Biotechnology, Physiology and Plant Improvement Unit, PO Box 812 Yaoundé, Cameroon. E-mail: youmbi_emmanuel@yahoo.fr

[5] 4 Avenue des Chasseurs, 34130 Saint Aunès, France. E-mail : fredbakry@gmail.com

✉ Corresponding author

Received
May 14, 2023

Accepted
October 25, 2023

How to cite
DZOYEM, C.U.D.; KAPUKU, L.I.; NDIANG, Z.; TCHABONG, G.R.N.; YOUMBI, E.; BAKRY, F. Suppression of female flowers and pollen fertility of male flowers of banana plants. **Pesquisa Agropecuária Brasileira**, v.58, e03376, 2023. DOI: https://doi.org/10.1590/S1678-3921.pab2023.v58.03376.

Supressão de flores femininas e fertilidade polínica de flores masculinas de bananeira

Resumo – O objetivo deste trabalho foi avaliar o efeito da supressão das flores femininas sobre a fertilidade do pólen das flores masculinas de bananeira. Dois modos de supressão das flores femininas, parcial e completo, foram aplicados logo após o florescimento em genótipos diploides, triploides e tetraploides de bananeira. Avaliou-se o efeito da supressão do florescimento sobre o número de flores masculinas e o comprimento das suas anteras, assim como a viabilidade do pólen e o tamanho e o número de grãos de pólen por antera. Testes de polinização foram realizados para avaliar o número de sementes obtidas da descendência de alguns dos genótipos. A supressão total de flores femininas aumenta significativamente a fertilidade polínica, estimada por meio do aumento do número de grãos de pólen por antera (até 80% por antera). A supressão total das flores femininas também melhora significativamente a produção de sementes nos cruzamentos (de 35 a 160% por cacho polinizado), o que sugere influência positiva da seiva que flui através do cacho na eficiência do pólen da bananeira.

Termos para indexação: *Musa*, genótipos, hibridização, ineficiência polínica, fluxo de seiva, semente.

Introduction

Most edible banana varieties result from numerous genetic crosses between two diploid wild seminiferous species, *Musa acuminata*

(genome A) and *Musa balbisiana* (genome B), either within or between species (De Langhe et al., 2010). While wild seminiferous banana plants are all diploid (2n=2x=22), cultivated varieties can also be diploid (especially in the centre of origin), however, mostly are triploid (2n=3x=33) and, very occasionally, tetraploid (2n=4x=44). These cultivars are the outcome of banana domestication resulting in major genetic changes such as interspecificity, polyploidy (represented by different proportions of A and/or B genomes), parthenocarpy and, above all, fruit sterility (Fuller et al., 2018). Indeed, during the domestication process, edible bananas have developed particularly high levels of male and female gametic sterility in combination with low capacity of fertilisation (Bakry & Horry, 2016), which is a major obstacle to the improvement of cultivated bananas (Aguilar Morán, 2013).

Wild (nonhybrid) banana genotypes that have retained their sexual reproductive system intact produce abundant fertile pollen, regardless of growing conditions. In contrast, the pollen fertility of cultivated bananas is much lower and also highly variable (Panda et al., 2019). This is thought to be the result of interactions between genetic background, environmental conditions, and plant physiology (Cenci et al., 2019). The physiological age of flowers also seems important, especially the flower stage at which pollen grains are collected (Ssebuliba et al., 2008). In addition, the ability of male sporophyte to provide nutrients (in the form of sap) to pollen during its development can be affected by growth conditions, which can influence the size, chemical composition, and performance of the pollen produced (Engelke et al., 2010). All these findings raise the question of whether a particular nutrient status might favour the reproductive performance of male pollen in edible bananas.

The inflorescence, which is vertical, pendant or sub-horizontal, has the peculiarity of having at first – on the same axis – the formation of female flowers which will later produce fruit and, then, a "male bud" which will continually produce male flowers, sometimes beyond the complete maturity of the bunch.

In industrial dessert banana plantations, the removal of the male bud is a common practice carried out a few days after the last bunch of fruit appears. This removal is made to improve the following parameters: the final dimensions of fruit, fruit filling, yield, and quality characteristics at harvest maturity (Balkic et al., 2016). Indeed, the male bud represent an important competing photosynthetic sink for developing banana fruit (Daniells et al., 1994). Access to photoassimilates is a priority for balanced plant development and optimum reproductive performance (Lemoine et al., 2013). Thus, it could be hypothesized that the required energy for fruit development, which is circulated as sap, can be redirected to be used exclusively for feeding the terminal part of the inflorescence bearing the male flowers.

The objective of this work was to evaluate the effect of the suppression of female flowers on the pollen fertility of male flowers of banana plants.

Materials and Methods

Male reproductive organs of the inflorescence were studied on ten distributed accessions (Table 1). The triploid cultivar Yangambi km5 (AAAcv - ITC: 1123) is known for its resistance to various diseases, and it is used as a progenitor in breeding programs. Both tetraploid improved varieties, FHIA 21 (AAABcv) and CRBP 39 (AAABcv), are disease resistant plants from breeding programs. In addition, the hybrid variety CARBAP 832 (AAABcv) was used as a female parent to test the biological fertility of pollen of the diploid clones.

The study was conducted at two experimental sites, in Mantem (at 508 m altitude), on the flank of the Mount Cameroon volcano, and Njombe (at 80 m altitude), on the coastal plain in the Fako and Moungo divisions of Cameroon. Both are characterized by tropical monsoon and trade-wind littoral climate (Am), according to world map of the Köppen-Geiger's climate classification. More specifically, Njombe (4°35'N, 9°39'E) has an average annual temperature of about 27°C, annual rainfall is estimated at 2,567 mm, and relative humidity varies between 60% and 99%. As for Mantem (4°50'N, 9°48'E), its average annual temperature is about 24°C, annual rainfall is estimated at 3,379 mm, and relative humidity varies between 64 and 99%. The soil of both sites is characterized by scoriaceous lava, with basalt blocks more or less concentrated on the surface, which is very stony and very gravelly.

The banana plants studied at the Njombe station are from the Musaceae reference collection of the African

Research Centre on Bananas and Plantains (CARBAP, Njombe, Cameroon). They were planted in blocks of 15 accessions. Each accession was identified by a single line consisting of five individuals. The studied accessions were distributed in four different blocks, which also grouped the genetic diversity of the banana plants conserved. At the Mantem station, planting was done in a split-plot design with complete randomization of accessions. Each accession was identified by a line of 10 individuals.

Two modalities were tested to assess the impact of female flower suppression on the male reproductive organs of the inflorescence (Figure 1). The first treatment (T1) was performed to suppress all female flowers in the first half of the bunch. The second treatment (T2) was performed for the suppression of all female flower on the flowering stem. The effect of these treatments was compared to that of the control plants that had no female flowers eliminated (T0). Suppression was done the day after the last row of female flowers appeared, using a knife previously cleaned with water and disinfected with 90° alcohol. The inflorescences were then bagged in a transparent white cotton bag, to allow wound healing and prevent possible infection by pathogens. After healing, only the male buds were bagged to protect them from pollen pollution.

The first observations of the male flowers started 10 days after the suppression of the female flowers. However, for the bunches that had not undergone any flower suppression (T0), observations were made after the appearance of the first cluster of male flowers. In the best case, observations were made until the male bud degenerated. The male flowers were evaluated for number of flowers, length of anthers, number of pollen grains per anther, pollen viability, pollen grain diameter, pollen fertility, and pollen efficiency.

The number of flowers was counted in all male flowers found at the daily opening of the bracts. If two bracts opened on the same day, the male flowers under each of them were counted separately. This parameter was assessed in the morning, between 6:00 and 8:00 h.

The length of anthers of male flowers was measured at the anthesis stage, using a magnifying glass and a vernier calliper. Measurements were made twice a week on 6 flowers according to their position on the male bud cushion: 2 flowers on the left side; 2 flowers in a central position; and 2 flowers on the right side.

The number of pollen grains per anther was measured at the anthesis, in the laboratory, from three different anthers taken from flowers in the left, middle and right position of the exposed bract. It consisted of the collection with a spatula all pollen grains present in an anther. These pollen grains were deposited in a uniform layer, on three separate slides containing a few drops of potassium iodide, for observation (X40) under the light microscope (Olesen & Warncke, 1989). All pollen grains were counted manually by moving the slides from right to left and from top to bottom to both ends. The sum of the pollen grains in the three slides gives the total amount of pollen per anther.

Pollen viability was assessed under a light microscope (X40) by differential staining, according to the description by Alexander (1969), to distinguish

Table 1. Genotypes and their locations in the study carried out in the Littoral region of Cameroon.

Accession	Ploidy and genome	Group	Origin	Accession number[(1)]	Study site	For more details see
Calcutta 4	2x (AA)	Wild	Indonesia	CMR00444	Njombe	Ongagna et al. (2020)
Khae Phrae	2x (AA)	Wild	Thailand	KHA	Njombe	Martin et al. (2020)
Balbisiana Cameroun	2x (BB)	Wild	Unknown	BCMR	Njombe	Ongagna et al. (2020)
Pisang Lilin	2x (AA)	Cultivar	Unknown	LILI	Njombe	Martin et al. (2020)
Tomolo	2x (AA)	Cultivar	Papua New Guinea	TOMO	Njombe	Bakry et al. (2007)
Mshale	2x (AA)	Cultivar	Highland of East Africa	CMR04165	Mantem	Waniale et al. (2021)
Mshare Mrerembo	2x (AA)	Cultivar	Highland of East Africa	CMR04167	Mantem	Perrier et al. (2019)
Pisang Madu	2x (AA)	Cultivar	Malaysia	MADU	Mantem	Martin et al. (2020)
Yangambi Km5	3x (AAA)	Cultivar	Congo (Kinshasa)	YGBI	Mantem	Fogain & Gowen (1998)
CARBAP 832	4x (AAAB)	Hybrid	Cameroon	CMR00446	Njombe	Dépigny et al. (2018)
FHIA 21	4x (AAAB)	Cultivar	Honduras	ITC1306	Mantem	Tenkouano et al. (2019)
CRBP 39	4x (AAAB)	Hybrid	Cameroon	CMR00451	Mantem	Noumbissié et al. (2016)

[(1)]Accession number available at Musa Germplasm Information System (2023).

aborted from nonaborted pollen. These observations were made twice a week throughout the life of the male inflorescence. In practice, pollen grains at the anthesis were collected from three different anthers and spread separately on three slides containing a few drops of dye. Each preparation was observed under four different fields. The average of the observations (12 in total) was used to determine a pollen viability rate (TVP, in percentage), according to the following formula: TVP (%) = (NPV×100)/(NPV+NPNV), where: NPV is the number of viable pollen; and NPNV is the number of nonviable pollen.

Pollen grain diameter was assessed on the same preparations, by measuring (X400) with a micrometric reticule inserted in the eyepiece. The average measurement is the result of three separate replicates of the measurement of the diameter of 40 pollen grains taken at the anthesis. This operation was carried out twice a week for the clones of the Mantem station throughout the life of the male inflorescence.

The pollen viability rates and the number of pollen grains per anther were used to determine the potential pollen fertility (PPF); this proportion was calculated according to the following formula: PPF = NP×TVP/100, where: FPP represents the number of potentially fertile pollen grains per anther; NP is the number of pollen; and TVP is the pollen viability rate.

The effect of flower suppression treatments on pollen efficiency was assessed in crosses, by quantifying the seed production of a tetraploid female parent (CARBAP 832), when pollinated by two edible diploid clones (Tomolo and Pisang Lilin) that had previously been subjected to both female flowers suppression treatments. The crosses were made at the Njombe Experimental Station, using the method described by Vuylsteke et al. (1997). Seed counts were carried out on mature fruit of "CARBAP 832". Seed were counted according to the position of fruit clusters (hands).

Descriptive and inferential statistical analyses were performed using the R software version 4.1.1. First,

Figure 1. Different modes of female flower suppressions on banana bunches: A, no suppression (T0); B, suppression of all female flowers in the first half of the bunch (T1); C, suppression of all female flowers on the flowering stem (T2). Photos by Gaetan Romaric Ngapmeu Tchabong.

an exploratory analysis was performed to search for possible differences between the modes of female flowers suppression on pollen fertility and, in case of significant differences, multiple comparison tests of the means were performed, at the 5% probability. In addition, the Dunnett's test was used to differentiate both modes of female flower suppressions, regarding their influence on the traits evaluated.

Results and Discussion

Pollen grains are uniformly stained in dark green with potassium iodide (KI), without distinguishing viable from nonviable pollen. Well individualized pollen grains are easy to count. When stained with the Alexander's dye, viable pollen grains show red/pink cytoplasm with a green pollen wall, and nonviable ones show completely dark green cytoplasm, or unstained with irregular contours affecting its morphology (Figure 2).

Wild banana male flowers are characterized both by the abundance of pollen grains and by their high pollen viability, which is near 100% for balbisiana Cameroon and Khae Phrae, and slightly lower for Calcutta 4 (94%) (Table 2). Compared to the viability of the wild genotypes, the pollen viability of the edible varieties is lower, but it does not decrease completely, it rarely decreases below 50% (Yangambi clone km5). Pisang Lilin is an exception, as it shows a very high pollen viability (95%) that is close to that of wild types. Our results are in line with those by Dodds (1943) and Ssebuliba et al. (2008). Pollen viability is indeed higher in monospecific varieties, in general, but especially in Pisang Lilin, which is also considered as polliniferous (Panda et al., 2019). Furthermore, *Musa balbisiana* is known for its high cytological capacity (viability and number of pollen grains per anther), in comparison with the seminiferous acuminata clones, although the pollen size is smaller (Fortescue & Turner, 2004).

The haploid pollen diameters of the wild accessions show homogeneous Gaussian distributions, with low coefficients of variation (Figure 3). The number of male flowers per node is highly variable, from about 26 for balbisiana Cameroun against 18 for Calcutta 4, and 8 only for Khae Phrae (Table 2). Balbisiana Cameroon also differs in that its pollen grains are slightly smaller in diameter (95 µm), and it has twice as many pollen grains per anther than those of the acuminata genotypes. However, there is no relationship between anther length and the amount of pollen they contain, as the anther length of balbisiana Cameroon is intermediate between the two acuminata (Table 2). Our results contrast with those by Oselebe et al. (2014), who showed that the longest anthers have a greater number of pollen. However, in our study, it is likely to show a genetic predisposition in pollen production of accessions (Fortescue & Turner, 2011) that is not

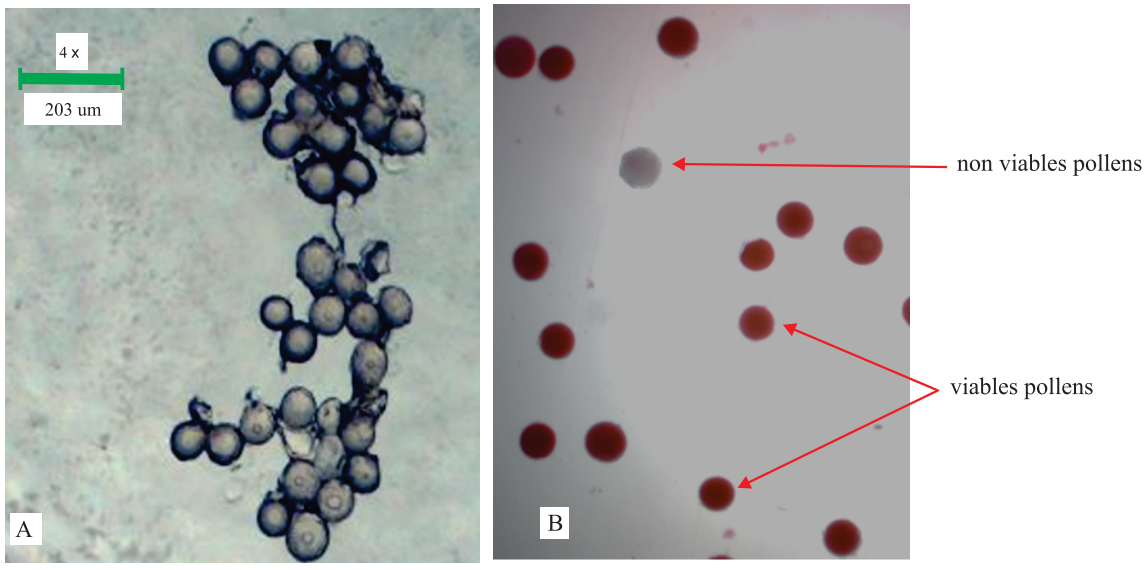

Figure 2. Microscopic observations of pollen grains after staining: A, potassium iodide (KI 2%); B, Alexander's dye.

affected by flower morphology. Pollen diameters are also much bigger, except for Tomolo and Pisang Madu. Globally, no significant differences were observed between the diameters of edible diploid and polyploid accessions. The curve distributions of these accessions tend to be more scattered and skewed than those for wild types (Figure 3), reflecting more heterogeneity in pollen production (Table 2). This diameter distribution (Table 3) suggests a significant proportion of 2N gametes in these pollen populations (Oselebe et al., 2014). In addition, an extension of the lowest modes was observed, suggesting variations of haploid micropore size, the largest one belonging to the Mlali group. The two tetraploid hybrids (CRBP 39 and FHIA 21) show

Table 2. Characteristics of pollen and male flowers in the studied banana genotypes[(1)].

Accession group	Genotype	Male flowers count	Anther length (mm)	Pollen size (µm)	Pollen number/ anther	Pollen viability rate (%)	Potential pollen fertility
Wild diploid	Balbisiana Cameroun	26±2a	18.5±1.5f	94.79±7.09i	9,127±1,493a	98.86±1.54a	9,012±1,384a
	Calcutta 4	18±3b	20.9±2.3d	98.62±9.52h	4,629±706b	94.01±5.23a	4,347±776c
	Khae Phrae	8±2j	14.8±1.7g	104.02±6.98g	4,890±1259b	97.02±3.61a	4,745±1,127bc
Cultivated diploid	Mshale	13±2gh	20.48±2.29e	134.40±16.43c	882±188e	71.21±10.79c	629±123fg
	Mshare Mrerembo	14±1fg	24.98±1.78c	127.11±16.67e	833±211e	72.08±15.29c	602±153fg
	Pisang Lilin	16±2d	15.17±1.67g	131.44±18.33d	1573±39d	95.05±0.75a	1,495±34e
	Pisang Madu	15±4e	18.30±1.79f	110.65±12.41f	2,872±788c	78.48±8.16b	2,277±661d
	Tomolo	12±1i	–	100.37±13.17h	377±19e	79.52±2.21b	300±18g
Triploid	Yangambi km5	18±2c	27.10±2.34b	148.35±20.60a	1,516±404d	48.88±8.25e	727±182f
Tetraploid	CRBP 39	12±1hi	24.77±2.17c	140.11±19.94b	3,911±1,055b	56.85±12.09d	2,174±619d
	FHIA 21	14±2f	28.97±2.56a	134.02±16.73c	8,238±1,869a	69.44±10.08c	5,624±1,100b

[(1)]Means followed by equal letters in the columns, do not differ, at 5% probability. Comparison was done by using Kruskall-Wallis' test in case of nonparametric data. Comparison was done by using SNK's test in case of parametric data, missing data.

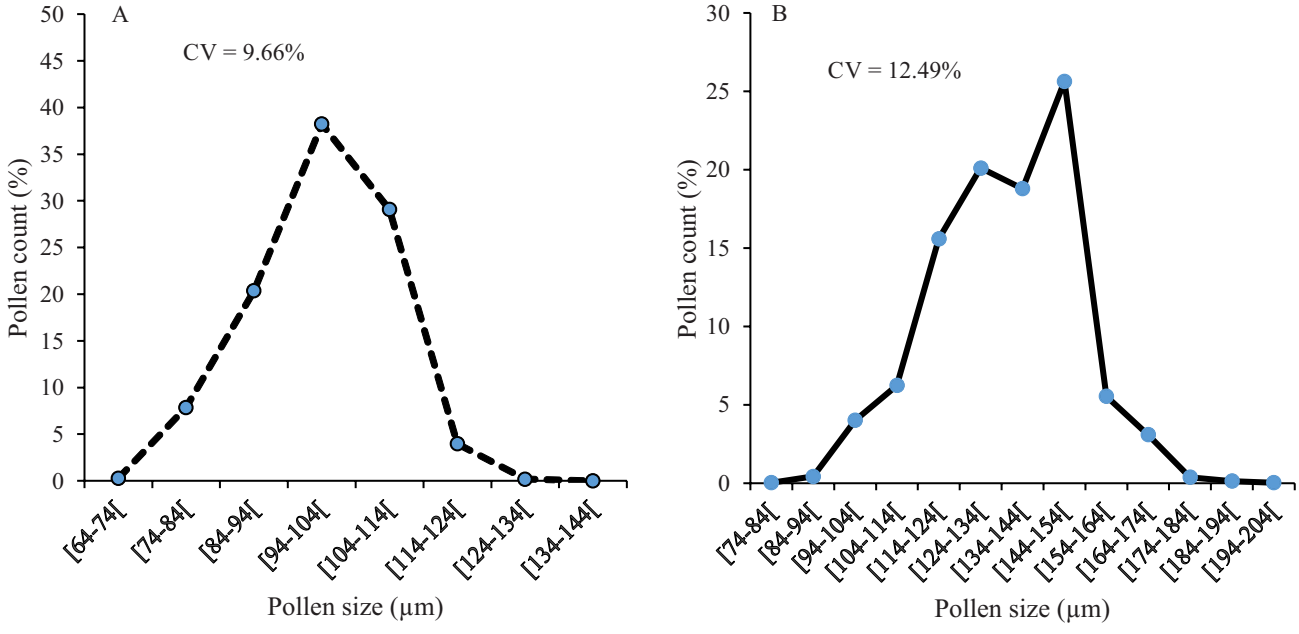

Figure 3. Monomodal (with mean-centred mode) and bimodal distribution of pollen diameters of Calcutta 4 wild (A) and FHIA 21 tetraploid hybrid (B) banana genotypes.

similar distributions, with slightly higher proportions of diploid pollen than those of haploid pollen. These latter results are consistent with the literature (Ortiz, 1997) on the ability of these tetraploid genotypes to produce diploid gametes and to obtain triploid progeny, when crossed with seminiferous diploids. For its part, the triploid Yangambi km5 (AAA) showed a trimodal distribution with haploid pollen in the first mode, a large majority of diploid pollen in the second mode and tetraploid pollen in the third mode is in the same proportion as the haploid pollen (Table 3). Overall, these results are not surprising, given the high proportion of irregular chromosome pairings in these genotypes (Tenkouano et al., 2011; Noumbissié et al., 2016; Baurens et al., 2019), which also affects pollen viability. It is likely that this pollen heterogeneity in edible bananas is responsible for their low pollen efficiency, which is commonly observed in banana breeding.

Anther length is also higher in edible varieties, especially in the polyploids. However, the number of pollen grains per anther is generally much lower than in wild clones, except for the tetraploid varieties CRBP 39 and FHIA 21, the latter of which carries a lot of pollen (Table 2). As for the wild genotypes, there is no proportional relationship between anther length and pollen content. These large quantities of viable pollen of tetraploid varieties, in proportions comparable to those of wild types, call for further studies on chromosome pairing and tetrad configuration at meiosis, to better understand our results at anthesis.

Partial (T1 treatment) or total (T2 treatment) flower suppression has little effect on the number of male flowers per node (Table 4). Sometimes this effect is positive (Pisang Madu, Yangambi km5), sometimes it is negative (Mshale, Mrerembo Mshare, Pisang Lilin, Tomolo, CRBP 39, FHIA 21) but without a major impact. The same is true for anther length, pollen grain diameter, and pollen viability. Variations, positive or negative depending on the clone, are observed in values from 1 to 5%, without ever exceeding 10%.

However, partial or total suppression of female flowers systematically increases the number of pollen grains per anther (Table 5). This increase is low (<10%) for the clone FHIA 21, which, like the wild clones, already contains a high amount of pollen without suppression. It is medium for the clones Pisang Lilin and Yangambi km5, high for the clones Pisang Madu and CRBP 39, and very high for the clone Tomolo, with a progression of almost 80% for the total suppression of female flowers. These results suggest a trophic constraint for these clones, which naturally

Table 3. Distribution of the number of pollen diameters according to banana genotypes studied by 10 µm interval classes[(1)].

Accession group and name	Study site	[64-74]	[74-84]	[84-94]	[94-104]	[104-114]	[114-124]	[124-134]	[134-144]	[144-154]	[154-164]	[164-174]	[174-184]	[184-194]	[194-204]	[204-214]	[214-224]	[224-234]	[234-244]	[244-254]	[254-264]	[264-274]	Total count
Seeded diploid																							
Calcutta 4	Njombe	3	85	220	413	314	43	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1,080
Khae Phrae	Njombe	-	4	19	148	165	23	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	360
Balbisiana Cameroun	Njombe	-	23	113	190	34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
Cultivated diploid																							
Tomolo	Njombe	3	48	270	333	152	101	30	15	5	2	1	-	-	-	-	-	-	-	-	-	-	960
Pisang Madu	Mamtem	8	49	722	2,272	2,224	2,503	1,083	263	103	10	3	-	-	-	-	-	-	-	-	-	-	9,240
Pisang Lilin	Njombe	-	3	17	85	165	472	414	465	178	145	45	30	10	8	2	0	0	0	0	1	-	2,040
Mshale	Mamtem	-	3	18	112	236	1,018	1,441	912	1,223	289	201	33	23	8	2	1	-	-	-	-	-	5,520
Mshare Mererembo	Mamtem	1	14	14	147	158	471	554	183	280	60	25	6	4	1	0	0	1	0	0	1	-	1,920
Cultivated triploid																							
Yangambi km5	Mamtem	-	1	8	78	149	764	1,526	1,462	2,968	1,049	1,498	376	329	158	33	22	13	3	2	0	1	10,440
Tetraploid hybrid																							
CRBP 39	Njombe	-	4	25	75	91	285	467	345	663	240	247	38	32	7	0	0	1	-	-	-	-	2,520
FHIA 21	Njombe	-	1	16	149	232	580	748	699	954	206	115	14	5	1	-	-	-	-	-	-	-	3,720

Shown in red are the modes of each pollen population; - missing data.

contain few pollen grains per anther (Daniells et al., 1994; Lemoine et al., 2013; De Schepper et al., 2013; Balkic et al., 2016; Jensen et al., 2016).

CARBAP 832 yields an average of 200 seed per bunch and up to 470 seed in a single bunch, when pollinated with a seedy fertility reference Calcutta 4. On the whole, the number of seed obtained per hand was quite regular from hand 1 to hand 6 (Table 6). In addition, the seed sets per hand were sufficiently homogeneous to show significant differences between the different treatments. Seed production is much lower in both diploid varieties. It varies greatly depending on the fertility of the pollen tested. When pollinated by untreated Pisang Lilin, CARBAP 832 produces an average of 49 seed per bunch and much less when pollinated by untreated Tomolo (an average of 2 seed per bunch). However, seed production increased when Pisang Lilin and Tomolo were treated, with 67 seed per bunch and 6 seed per bunch, respectively, for T2, and 57 seed per bunch and 3 seed per bunch, respectively, for T1. These results, although modest in absolute values, translated for the T2 treatment into an increase of +35% for Pisang Lilin and more than +160% for Tomolo, even if for the latter, the result of 6 seed per bunch remains very low. Thus, there is a proportional relationship between the number of suppressed female flowers and pollen efficiency. This result is perfectly related to the variable "number of pollen grains

Table 4. Influence of flower suppression on pollen and male flowers of diploid and polyploid bananas. The means of the T0 treatments are presented. Treatments T1 and T2 show the positive (+) and negative (-) difference between the means of the fruit ablation modes and the corresponding control for each parameter(1).

Accession group	Genotype	Mode of flower suppression	Male flowers count	Anther lenght (mm)	Pollen size (µm)	Pollen number/ anther.	Pollen viability rate (%)	Potential pollen fertility
Cultivated diploid	Mshale	T0	13.14	20.48	134.40	881.50	71.21	629.27
		T1	-1.36***	0.2	-5.66***	+298***	+5.23***	+284***
		T2	-1.19***	0.4	-0.85	+270***	+2.27***	+211***
	Mshare Mrerembo	T0	13.95	24.98	127.11	833.11	72.08	601.72
		T1	-2.0***	-2.5***	+2.78***	+131***	-11.97***	-19
		T2	-2.33***	-1.9***	+4.79***	+237***	-4.82***	114
	Pisang Lilin	T0	15.98	15.17	131.50	1573	95.05	1495
		T1	-0.136	+2.56***	-4.55***	*118.70***	-0.32	+107.68***
		T2	-0.004	+1.15***	1.37	+263.85***	+0.82***	+265.89***
	Pisang Madu	T0	15.45	18.3	110.65	2871.61	78.48	2276.65
		T1	+1.23***	+1.4***	-0.55*	+344***	+1.86***	223
		T2	+2.6***	+2.0***	0.43	+1322***	+7.81***	+1255***
	Tomolo	T0	12.07	–	100.37	377	79.52	300
		T1	-0.03	–	–	+53.89***	-0.32	+107.68***
		T2	-0.16*	–	–	+300.85***	+0.81***	+265.89***
Triploid	Yangambi km5	T0	17.55	27.1	148.35	1515.75	48.88	727.01
		T1	0.23	0.2	+1.15***	+251***	+2.13***	+135***
		T2	0.09	+1.7***	+1.30***	+413***	+5.88***	+309***
Tetraploid	CRBP 39	T0	12.29	24.77	140.11	3911.32	56.85	2173.80
		T1	-7.0***	0.03	-6.48***	499	+13.13***	+909**
		T2	-2.0***	+2.2***	-2.37***	+2060***	-4.26***	+971***
	FHIA 21	T0	14.22	28.97	134.02	8237.51	69.44	5624.34
		T1	-2.0***	-2.9***	-1.33**	-1117***	-0.60	-855*
		T2	-2.0***	-1.1***	-0.21	+594*	-0.32	381

***, **, * Significant at 0.1, 1, and 5% probability, respectively. Comparison was done by using the Dunnett's test. –, missing data.

Table 5. Assessing the effect of suppressing total fruit in the bunch on the number of pollen per anther[1].

Genotype	Number of pollen per anther T0→T2	Effect of total flower suppression	
Tomolo	377→ +300	80%	Very high
Mshale	881→ + 270	30%	Medium
Mshare Mrerembo	833→ +237	28%	Medium
Yangambi km5	1,515→ +413	27%	Medium
Pisang Lilin	1,573→ +263	17%	Medium
Pisang Madu	2,871→ +1,322	46%	High
CRBP 39	3,911→ +2,060	53%	High
FHIA 21	8,327→ +594	7%	Low

[1]Low, effect < 10%; medium, 10%<effect<30%; high, 30%<effect<60%; very high, effect> 60%. →, Direction of increase of the number of pollen grains per anther.

Table 6. Biological efficiency of pollen in crossbreeding with CARBAP 832 after the suppression of female flowers of Pisang Lilin and Tomolo[1].

Genotype	Treatment	Number of cross	Mean seed set per hand						Mean±standard deviation
			Hand 1	Hand 2	Hand 3	Hand 4	Hand 5	Hand 6	
Pisang Lilin	T0	9	49	46	47	48	55	50	49.35±4.38c
	T1	9	60	55	58	59	56	53	56.63±6.67b
	T2	9	65	66	64	68	68	69	66.67±5.00a
Tomolo	T0	9	3	2	3	2	1	2	2.28±0.81c
	T1	9	2	3	4	3	2	3	2.94±1.45b
	T2	9	6	6	7	5	7	6	6.11±1.54a

[1]Means followeb by equal letters, in the column by genotype, do not differ by Kruskall-Wallis's test, at 5% probability.

per anther", which could therefore be an essential component of pollen fertility. It is also possible that this "number of pollen grains per anther" variable is only one component of the response to the treatments, and that the suppression of female flowers may also have the physiological effect of "energizing" the pollen grains, for instance, by increasing the number of starch grains per microspore. Unfortunately, experimental conditions did not allow to examine this variable. Finally, there is a significant influence of the sap availability in the bunch on banana pollen efficiency, irrespective of pollen viability rates, which varied little according to the suppression treatments applied.

Conclusions

1. The quantity of pollen grains in the anthers increases after the suppression of female flowers of banana (*Musa* spp.) plants.

2. The suppression of female flowers from the male progenitors has a beneficial and significant effect on the improvement of the fertility of banana pollen and on the number of seed in crosses, especially when the male progenitor produces very few viable pollen.

Acknowledgments

To Plantations du Haut-Penja, a subsidiary of the French company "Compagnie Fruitière", for their logistical support and for providing the land that enabled the experiments to be carried out on the Mantem site; to Centre Africain de Recherches sur Bananiers et Plaintain (CARBAP), for providing the plant material and technical equipment for the activities.

References

AGUILAR MORÁN, J.F. Improvement of Cavendish banana cultivars through conventional breeding. **Acta Horticulturae**, v.986, p.205-208, 2013. DOI: https://doi.org/10.17660/ActaHortic.2013.986.21.

ALEXANDER, M.P. Differential staining of aborted and non-aborted pollen. **Stain Technology**, v.44, p.117-122, 1969. DOI: https://doi.org/10.3109/10520296909063335.

BAKRY, F.; HORRY, J.-P. Advances in genomics: applications to banana breeding. **Acta Horticulturae**, v.1114, p.171-180, 2016. DOI: https://doi.org/10.17660/ActaHortic.2016.1114.24.

BAKRY, F.; REBERDIERE, N.P. de la; PICHOT, S.; JENNY, C. In liquid medium colchicine treatment induces non chimerical doubled-diploids in a wide range of mono- and interspecific diploid banana clones. **Fruits**, v.62, p.3-12, 2007. DOI: https://doi.org/10.1051/fruits:2006043.

BALKIC, R.; GUNES, E.; ALTINKAYA, L.; GUBBUK, H. Effect of male bud flower removal on yield and quality of 'Dwarf Cavendish' banana. **Acta Horticulturae**, v.1139, p.587-590, 2016. DOI: https://doi.org/10.17660/ActaHortic.2016.1139.101.

BAURENS, F.-C.; MARTIN, G.; HERVOUET, C.; SALMON, F.; YOHOMÉ, D.; RICCI, S.; ROUARD, M.; HABAS, R.; LEMAINQUE, A.; YAHIAOUI, N.; D'HONT, A. Recombination and large structural variations shape interspecific edible bananas genomes. **Molecular Biology and Evolution**, v.36, p.97-111, 2019. DOI: https://doi.org/10.1093/molbev/msy199.

CENCI, A.; HUEBER, Y.; ZORRILLA-FONTANESI, Y.; VAN WESEMAEL, J.; KISSEL, E.; GISLARD, M.; SARDOS, J.; SWENNEN, R.; ROUX, N.; CARPENTIER, S.C.; ROUARD, M. Effect of paleopolyploidy and allopolyploidy on gene expression in banana. **BMC Genomics**, v.20, art.244, 2019. DOI: https://doi.org/10.1186/s12864-019-5618-0.

DANIELLS, J.W.; LISLE, A.T.; BRYDE, N.J. Effect of bunch trimming and leaf removal at flowering on maturity bronzing, yield, and other aspects of fruit quality of bananas in North Queensland. **Australian Journal of Experimental Agriculture**, v.34, p.259-265, 1994. DOI: https://doi.org/10.1071/EA9940259.

DE LANGHE, E.; HŘIBOVÁ, E.; CARPENTIER, S.; DOLEŽEL, J.; SWENNEN, R. Did backcrossing contribute to the origin of hybrid edible bananas? **Annals of Botany**, v.106, p.849-857, 2010. DOI: https://doi.org/10.1093/aob/mcq187.

DE SCHEPPER, D.; DE SWAEF, T.; BAUWERAERTS, I.; STEPPE, K. Phloem transport: a review of mechanisms and controls. **Journal of Experimental Botany**, v.64, p.4839-4850, 2013. DOI: https://doi.org/10.1093/jxb/ert302.

DÉPIGNY, S.; NOUPADJA, P.; TOMEKPE, K.; BONNEVIOT, P.; LESCOT, T.; BAKRY, F. 'CARBAP K74': a triploid plantain-like hybrid designed to promote sustainable plantain-based cropping systems. **Acta Horticulturae**, v.1196, p.63-70, 2018. DOI: https://doi.org/10.17660/ActaHortic.2018.1196.7.

DODDS, K.S. Genetical and cytological studies of Musa: V. Certains edible diploids. **Journal of Genetics**, v.45, p.113-139, 1943. DOI: https://doi.org/10.1007/BF02982931.

ENGELKE, T.; HIRSCHE, J.; ROITSCH, T. Anther-specific carbohydrate supply and restoration of metabolically engineered male sterility. **Journal of Experimental Botany**, v.61, p.2693-2706, 2010. DOI: https://doi.org/10.1093/jxb/erq105.

FOGAIN, R.; GOWEN, S.R. "Yangambi km5" (Musa AAA, Ibota subgroup): a possible source of resistance to *Radopholus similis* and *Pratylenchus goodeyi*. **Fundamental and Applied Nematology**, v.21, p.75-80, 1998.

FORTESCUE, J.A.; TURNER, D.W. Pollen fertility in *Musa*: viability in cultivars grown in Southern Australia. **Australian Journal of Agricultural Research**, v.55, p.1085-7091, 2004. DOI: https://doi.org/10.1071/AR04078.

FORTESCUE, J.A.; TURNER, D.W. Reproductive Biology. In: PILLAY, M.; TENKOUANO, A. (Ed.). **Banana breeding**: progress and challenges. Boca Raton: CRC Press, 2011. p.145-176.

FULLER, T.L.; SESINK CLEE, P.R.; NJABO, K.Y.; TRÓCHEZ, A.; MORGAN, K.; MEÑE, D.B.; ANTHONY, N.M.; GONDER, M.K.; ALLEN, W.R.; HANNA, R.; SMITH, T.B. Climate warming causes declines in crop yields and lowers school attendance rates in Central Africa. **Science of The Total Environment**, v.610/611, p.503-510, 2018. DOI: https://doi.org/10.1016/j.scitotenv.2017.08.041.

JENSEN, K.H.; BERG-SØRENSEN, K.; BRUUS, H.; HOLBROOK, N.M.; LIESCHE, J.; SCHULZ, A.; ZWIENIECKI, M.A.; BOHR, T. Sap flow and sugar transport in plants. **Reviews of Modern Physics**, v.88, 2016. DOI: https://doi.org/10.1103/RevModPhys.88.035007.

LEMOINE, R.; LA CAMERA, S.; ATANASSOVA, R.; DÉDALDÉCHAMP, F.; ALLARIO, T.; POURTAU, N.; BONNEMAIN, J.-L.; LALOI, M.; COUTOS-THÉVENOT, P.; MAUROUSSET, L.; FAUCHER, M.; GIROUSSE, C.; LEMONNIER, P.; PARRILLA, J.; DURAND, M. Source-to-sink transport of sugar and regulation by environmental factors. **Frontiers in Plant Science**, v.4, art.272, 2013. DOI: https://doi.org/10.3389/fpls.2013.00272.

MARTIN, G.; CARDI, C.; SARAH, G.; RICCI, S.; JENNY, C.; FONDI, E.; PERRIER, X.; GLASZMANN, J.C.; D'HONT, A.; YAHIAOUI, N. Genome ancestry mosaics reveal multiple and cryptic contributors to cultivated banana. **The Plant Journal**, v.102, p.1008-1025, 2020. DOI: https://doi.org/10.1111/tpj.14683.

MUSA GERMPLASM INFORMATION SYSTEM. Available at: <https://www.crop-diversity.org/mgis/accession-search>. Accessed on : May 25 2023.

NOUMBISSIÉ, G.B.; CHABANNES, M.; BAKRY, F.; RICCI, S.; CARDI, C.; NJEMBELE, J.-C.; YOHOUME, D.; TOMEKPE, K.; ISKRA-CARUANA, M.-L.; D'HONT, A.; BAURENS, F.-C. Chromosome segregation in an allotetraploid banana hybrid (AAAB) suggests a translocation between the A and B genomes and results in eBSV-free offsprings. **Molecular Breeding**, v.36, art.38, 2016. DOI: https://doi.org/10.1007/s11032-016-0459-x.

OLESEN, J.M.; WARNCKE, E. Temporal changes in pollen flow and neighbourhood structure in a population of *Saxifraga hirculus* L. **Oecologia**, v.79, p.205-211, 1989. DOI: https://doi.org/10.1007/BF00388480.

ONGAGNA, A.; DZOKOUO DZOYEM, C.U.; YOUMBI, E.; BAKRY, F. Positive effect of *in vitro* embryo rescue on breaking the dormancy of wild banana seeds compared to direct sowing in the greenhouse. **Fruits**, v.75, p.194-203, 2020. DOI: https://doi.org/10.17660/th2020/75.5.2.

ORTIZ, R. Occurrence and inheritance of 2n pollen in *Musa*. **Annals of Botany**, v.79, p.449-453, 1997. DOI: https://doi.org/10.1006/anbo.1996.0367.

OSELEBE, H.O.; NNAMANI, C.V.; IKEH, E. Pollen diversity, viability and floral structure of some *Musa* genotypes. **Nigerian Journal of Biotechnology**, v.27, p.21-27, 2014.

PANDA, A.K.; SOORIANATHASUNDARAM, K.; VIJAYAKUMAR, R.M. Screening for male fertility status in selected banana genotypes. **Electronic Journal of Plant Breeding**, v.10, p.1309-1316, 2019. DOI: https://doi.org/10.5958/0975-928X.2019.00167.4.

PERRIER, X.; JENNY, C.; BAKRY, F.; KARAMURA, D.; KITAVI, M.; DUBOIS, C.; HERVOUET, C.; PHILIPPSON, G.; DE LANGHE, E. East African diploid and triploid bananas: a genetic complex transported from South-East Asia. **Annals of Botany**, v.123, p.19-36, 2019. DOI: https://doi.org/10.1093/aob/mcy156.

SSEBULIBA, R.N.; TENKOUANO, A.; PILLAY, M. Male fertility and occurrence of 2n gametes in East African Highland bananas (Musa spp.). **Euphytica**, v.164, p.53-62, 2008. DOI: https://doi.org/10.1007/s10681-007-9637-6.

TENKOUANO, A.; LAMIEN, N.; AGOGBUA, J.; AMAH, D.; SWENNEN, R.; TRAORÉ, S.; THIEMELE, D.; ABY, N.; KOBENAN, K.; GNONHOURI, G.; YAO, N.; ASTIN, G.; SAWADOGO-KABORE, S.; TARPAGA, V.; ISSA, W.; LOKOSSOU, B.; ADJANOHOUN, A.; AMADJI, G.L.; ADANGNITODE, S.; IGUE, K.A.D.; ORTIZ, R. Promising high-yielding tetraploid plantain-bred hybrids in West Africa. **International Journal of Agronomy**, v.2019, art.3873198, 2019. DOI: https://doi.org/10.1155/2019/3873198.

TENKOUANO, A.; PILLAY, M.; ORTIZ, R. Breeding techniques. In: PILLAY, M.; TENKOUANO, A. (Ed.). **Banana breeding**: progress and challenges. Boca Raton: CRC Press, 2011. p.181-198.

VUYLSTEKE, D.; ORTIZ, R.; FERRIS, R.S.B.; CROUCH, J.H. Plantain Improvement. In: JANICK, J. (Ed.). **Plant Breeding Reviews**. IITA Books and Book Chapters. New York: John Wiley & Sons, 1997. v.14, p.267-320. DOI: https://doi.org/10.1002/9780470650073.ch9.

WANIALE, A.; SWENNEN, R.; MUKASA, S.B.; TUGUME, A.K.; KUBIRIBA, J.; TUSHEMEREIRWE, W.K.; BATTE, M.; BROWN, A.; TUMUHIMBISE, R. Seed set patterns in East African highland cooking bananas show asymmetric distribution in bunches and fruits. **Agronomy**, v.11, art.763, 2021. DOI: https://doi.org/10.3390/agronomy11040763.